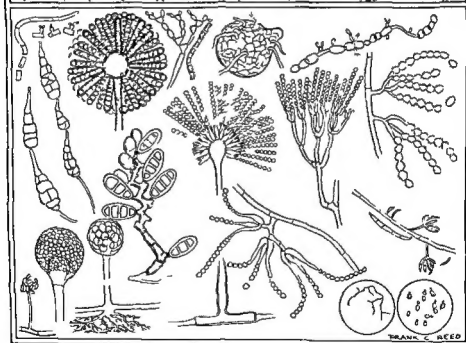
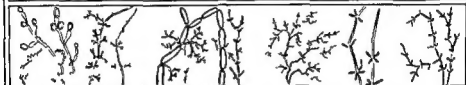
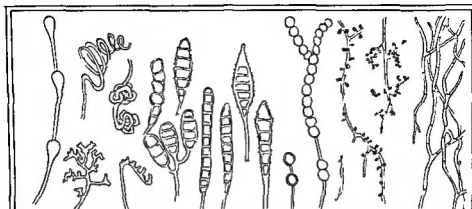


Laboratory Identification
of
Pathogenic Fungi Simplified

Publication Number 370
AMERICAN LECTURE SERIES ©

A Monograph in
The BANNERSTONE DIVISION of
AMERICAN LECTURES IN TESTS AND TECHNIQUES

Edited by
GILBERT DALLDORF M.D.



(Second Edition)

Laboratory Identification of Pathogenic Fungi Simplified

By

ELIZABETH L. HAZEN, PH.D.

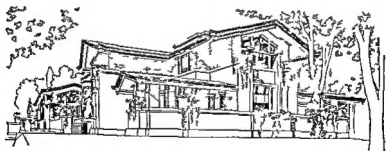
Associate Bacteriologist (Mycology)

and

FRANK CURTIS REED

Laboratory Illustrator and Photographer

*Division of Laboratories and Research
New York State Department of Health
Albany, New York*



CHARLES C. THOMAS • PUBLISHER
Springfield • Illinois • USA

CHARLES C THOMAS PUBLISHER
BANNERSTONE HOUSE
301 327 East Lawrence Avenue Springfield Illinois USA

Published simultaneously in the British Commonwealth of Nations by
BLACKWELL SCIENTIFIC PUBLICATIONS LTD OXFORD ENGLAND

Published simultaneously in Canada by
THE RYERSON PRESS TORONTO

This monograph is protected by copyright. No
part of it may be reproduced in any manner
without written permission from the publisher

Copyright 1955 and 1960 by CHARLES C THOMAS PUBLISHER

Library of Congress Catalog Card Number 59 14109

With THOMAS BOOKS careful attention is given to all details of
manufacturing and design. It is the Publisher's desire to present books
that are satisfactory as to their physical qualities and artistic possibilities
and appropriate for their particular use. THOMAS BOOKS will be true
to those laws of quality that assure a good name and good will.

To the Memory of
DR RHODA W BENHAM

1894—1957

*who was a constant source
of inspiration and encouragement*

Preface to the Second Edition

THE OBJECTIVE of the second edition remains the same as that of the first—in aid in the teaching of the essentials in the identification of pathogenic fungi to the beginner and as a bench companion for the bacteriologist engaged in mycologic diagnosis.

Many additions and substitutions have been made in the hope of increasing the usefulness of the monograph. A section has been added on the contaminants commonly encountered in the laboratory and so often mistaken for pathogenic fungi by the inexperienced. The colonial and microscopic characteristics by which these saprophytic fungi can be identified and differentiated from the pathogen are described and illustrated photographically.

In the section on the pathogenic fungi incitants of South American blastomycosis, aspergillosis, mucormycosis, and moniliasis incited by species of *Candida* other than *Candida albicans* are described. South American blastomycosis is not endemic in North America but travel between the two continents is so common that the possibility of infection in persons who have been in South America should not be overlooked. The systemic form of aspergillosis and mucormycosis has increased since the advent of the broad spectrum antibiotics, steroids, and folic acid antagonists.

Drawings of characteristic microscopic structures of some of the common fungi constitute a new frontispiece which should be useful to the student in testing his ability to identify these fungi. Some of the photomicrographs have been replaced with better prints. Recent references have been included in the bibliography and new formulae have been added to the list of media.

Again we acknowledge with deep appreciation the valuable assistance of our many colleagues without which this second edition would not have been possible.

E L H
F C R

Preface to the First Edition

THIS MONOGRAPH is the outgrowth of an experience in teaching diagnostic mycologic methods to students who had been trained in bacteriologic procedures but who had little or no experience in the field of mycology. Some students were assistants in this laboratory, some were visitors from other laboratories, and some were physicians preparing to direct local laboratories in New York State. All wished to become familiar in a short time with the essential procedures and criteria in the identification of the incitants of fungus infections.

As a visual aid in this program of instruction the exhibit panel shown in the *Frontispiece* was designed to present the characteristic features upon which the identification of the pathogenic fungi is based. Giant living colonies and diagrammatic drawings of the microscopic structures familiarized the students with the morphology of the fungi. The exhibit was also used effectively in lecture demonstrations to medical students. Later as a matter of convenience photographs of the individual sections of the exhibit were bound with a supplementary text as a manual for use at the laboratory bench. Impetus to make the manual more widely available came from the response of members of the New York State Association of Public Health Laboratories to whom it was shown at the Annual Meeting in 1952. In the present book photomicrographs replace the drawings of the original exhibit. Tabular and other textual descriptive information has been added together with formulae of the essential culture media and a selective list of references. The objective remains the same as in the earlier presentations, namely, an aid to the teaching of the essentials in the identification of the pathogenic fungi to the beginner and a bench companion for the bacteriologist engaged in mycologic diagnosis.

Only those pathogenic fungi commonly encountered in North America are included in this work and all illustrations are from cultures studied in this laboratory. The pathogenic fungi are, with few exceptions members of the class of Fungi Imperfecti that is fungi in which the sexual spore has not been demonstrated. Identification is based upon asexual spores the conidia, which are borne on specialized hyphae (conidiophores). The diseases caused by the pathogenic fungi have been roughly classified into superficial and systemic or deep seated mycoses.

In publishing this monograph our appreciation goes to the students whose responses have helped us to sharpen the various presentations. Acknowledgment is also made to our colleagues whose encouragement and valuable assistance are responsible for the decision to offer to others this outline of a practical laboratory experience.

E L H
F C R

Contents

	Page
<i>Preface to the Second Edition</i>	ix
<i>Preface to the First Edition</i>	xi
SUPERFICIAL MYCOSES (DERMATOPHYTOSSES (RING WORM))	3
<i>Microsporum</i>	5
<i>Microsporum audouinii</i>	6
<i>Microsporum canis</i> (<i>Microsporum lanosum</i> <i>Microsporum felinum</i>)	10
<i>Microsporum gypsum</i> (<i>Microsporum fulvum</i>)	14
<i>Trichophyton</i>	19
<i>Trichophyton mentagrophytes</i> (<i>Trichophyton gypsum</i>)	20
<i>Trichophyton rubrum</i> (<i>Trichophyton purpureum</i>)	24
<i>Trichophyton tonsurans</i> (<i>Trichophyton crateriforme</i>)	25
<i>Trichophyton verrucosum</i> (<i>Trichophyton fauiforme</i>)	32
<i>Trichophyton schoenleii</i> (<i>Achorion schoenleii</i>)	36
<i>Trichophyton violaceum</i>	40
<i>Epidermophyton</i>	
<i>Epidermophyton floccosum</i> (<i>Epidermophyton inguinale</i> <i>Epidermophyton cruris</i>)	44
DEEP SEATED MYCOSES (SUBCUTANEOUS SYSTEMIC)	49
Actinomycosis	51
<i>Actinomyces bovis</i>	
Nocardiosis	55
<i>Nocardia asteroides</i>	
Blastomycosis—North American	59
<i>Blastomyces dermatitidis</i>	
Blastomycosis—South American	62
<i>Paracoccidioides brasiliensis</i>	
Moniliasis	67
<i>Candida albicans</i>	
Coccidioidomycosis	73
<i>Coccidioides immitis</i>	

	<i>Page</i>
Cryptococcosis	77
<i>Cryptococcus neoformans</i>	
Geotrichosis	81
<i>Geotrichum candidum</i>	
Histoplasmosis	85
<i>Histoplasma capsulatum</i>	
Aspergillosis	88
<i>Aspergillus fumigatus</i>	
Systemic Mucormycosis	92
Mucormycosis	97
<i>Monosporium apiospermum</i>	
<i>Allecheria boydii</i>	
Sporotrichosis	101
<i>Sporotrichum schenckii</i> (<i>Sporotrichum beurmanni</i>)	
Chromoblastomycosis	105
<i>Phialophora verrucosa</i>	
<i>Fonsecaea pedrosoi</i>	
<i>Fonsecaea compacta</i>	
<i>Fonsecaea dermatitidis</i>	
<i>Cladosporium carrionii</i>	
CONTAMINANTS	115
<i>Penicillium</i> sp	116
<i>Paecilomyces</i> sp	116
<i>Scopulariopsis</i> sp	116
<i>Cephalosporium</i> sp	115
<i>Trichosporon</i> sp	120
<i>Rhodotorula</i> sp	120
<i>Monilia sitophila</i>	122
<i>Streptomyces</i> sp	122
<i>Fusarium</i> sp	124
<i>Cladosporium</i> (<i>Hormodendrum</i>) sp	124
<i>Phoma</i> sp	126
<i>Pullularia</i> sp	126
<i>Helminthosporium</i> sp	125
<i>Alternaria</i> sp	125

	<i>Page</i>
<i>Aspergillus</i> sp	130
<i>Syncephalastrum</i> sp	130
<i>Mucor</i> sp	132
<i>Rhizopus</i> sp	132
MEDIA	135
References	139

Laboratory Identification
of
Pathogenic Fungi Simplified

Aided by a grant from the Brown Hazen Fund

Superficial Mycoses

Dermatophytoses (Ringworm)

THE SUPERFICIAL MYCOSES are the most common and widely distributed of all fungus diseases. They are confined to the keratinized layers of skin and its appendages but nevertheless are of major importance since they are so widespread and may cause great discomfort and even at times are very disabling.

These diseases are incited by a group of fungi, the dermatophytes, embracing many species. The isolation and identification of the fungus from scrapings from skin lesions, infected nails, or from stubs of broken hairs are essential to specific diagnosis.

This group of fungi is represented by three genera based upon the type of macroconidia (fuseaux) formed: *Microsporum*, *Trichophyton*, and *Epidermophyton*.

Note. The dermatophytes are usually easily isolated on Sabouraud's glucose agar at room temperature; however, in cases of grossly contaminated specimens a selective isolation agar, Mycosel (Baltimore Biological Laboratory, Inc.) should be used.

MICROSPORIUM

THE GENUS *Microsporum* consists of three commonly recognized species *M. audouinii*, *M. canis* and *M. gypsum*. They attack hair and glabrous skin. These fungi are the chief incitants of ring worm of the scalp (*tinea capitis*) among children in the United States. The infected hair shows a sheath of spores in the form of a mosaic about the hair shaft (ectothrix type) and under filtered ultraviolet light (Wood's lamp) there is a brilliant green fluorescence. In the infected skin segmented branching mycelium is found.

They form cottony or downy matted or powdery aerial mycelium and vary in color from white to grayish white or buff to various shades of brown.

They produce characteristic large thick rough or smooth walled multiseptate spindle shaped macroconidia (*fuseaux*) and small single celled clavate spores attached directly or to short sterigmata on the sides of the hyphae. Pectinate hyphae, nodular bodies, racquet hyphae and chlamydospores are also formed.

MICROSPORUM AUDOUINI

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Flat grayish white velvety aerial
mycelium (with button in center)
rose brown pigment on under
surface

Polished rice
grains

No aerial mycelium brownish disc
coloration of grains

MICROSCOPIC

Corn meal agar

Pectinate hyphae

Honey agar plus
yeast extract (10
mg/ml)

Microconidia microconidia

Incubated at 25 C

MICROSPORUM AUDOUINI

THE SPECIES *Microsporum audouini* is of human origin and is the chief agent of tinea capitis among children in the United States. This dermatophyte also attacks glabrous skin.

Cultural and Microscopic Characteristics

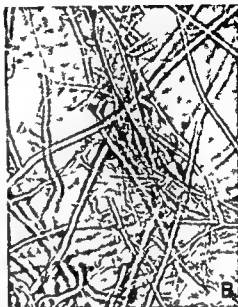
The colony on Sabouraud's glucose agar is slow growing, flat with a button in the center and a surface of grayish white, closely matted, velvety, sometimes fluffy; aerial mycelium occasionally showing radial foldings; a rose brown pigment on the under surface.

Microscopically large, thick, rough or smooth walled, multi-septate, spindle shaped macroconidia (fuseaux) and numerous single celled, clavate microconidia and pectinate hyphae may be seen. The macroconidia can seldom be demonstrated on Sabouraud's glucose medium. An enriched medium is required.

MICROSPORUM AUDOUINI

Figure 1

- A Colony on Sabouraud's glucose agar after three weeks
- B Honey agar plus wet extract slide culture preparation showing multiseptate spindle shaped microconidia and clavate microconidia $\times 400$
- C Culture mount from corn meal agar showing pectinate hyphae $\times 400$
- D Infected hair with small spores forming sheath around hair $\times 400$



MICROSPORUM CANIS

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose agar	White cottony or wooly aerial mycelium becoming powdery with age bright yellow to orange pigment on undersurface
Polished rice grains	Grains covered with heavy white cottony aerial mycelium becoming powdery with age pinkish buff discoloration of medium

MICROSCOPIC

Sabouraud's glucose agar	Microconidia microconidia
Polished rice grains	Microconidia microconidia

MICROSPORUM CANIS

(*Microsporum lanosum* *Microsporum felineum*)

THE SPECIES *Microsporum canis* is of animal origin and is responsible for roughly 10 per cent of tinea capitis among children in the United States. This fungus also attacks glabrous skin.

Cultural and Microscopic Characteristics

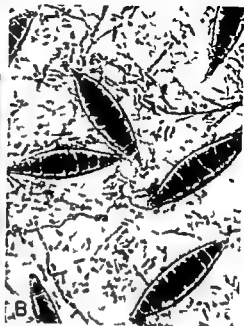
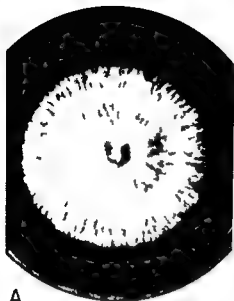
The colony on Sabouraud's glucose agar develops fairly rapidly, forming a white, cottony or wooly aerial mycelium becoming powdery with a central depressed area; sometimes showing radial folds. The pigment on the undersurface is yellowish orange, later changing to reddish brown.

Microscopically, numerous large, thick, rough-walled, multi-septate, spindle-shaped macroconidia (fuseaux) and numerous clavate or elongated microconidia are found. Racquet hyphae and chlamydospores are also present.

MICROSPORIUM CANIS

Figure 2

- A Colony on Sabouraud's glucose agar after three weeks
- B Slide culture preparation stained with lactophenol cotton blue showing numerous macroconidia and small single celled microconidia
X 600
- C Multiseptate spindle shaped microconidia X 400
- D Young macroconidia attached to hyphal branches X 400
- E Racquet hypha X 400



MICROSPORUM Gypseum

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Powdery aerial mycelium of buff
to light brown or cinnamon
color

Polished rice grains

Grains covered with powdery aerial
mycelium of pinkish cinnamon
color

MICROSCOPIC

Sabouraud's glucose
agar

Microconidia

Polished rice grains

Microconidia

MICROSPORIUM GYPSEUM

(Microsporum fulvum)

THE SPECIES *Microsporum gypseum* long considered to be of animal origin has more recently been found to be a soil inhabiting fungus. Of the three microspora it is least commonly encountered as the cause of tinea capitis or ringworm of the glabrous skin.

Cultural and Microscopic Characteristics

The colony is comparatively fast growing, shows a central cottony boss surrounded by a flat powdery cinnamon brown mycelium terminating in a border of downy white mycelium. The pigment on the undersurface is reddish brown.

Microscopically, numerous large rough thick walled multi-septate spindle shaped macroconidia with slightly rounded ends are found.

MICROSPORIUM CYPRIUM

Figure 3

A Colony on Sabouraud's glucose agar after three weeks

B Numerous large rough thick walled multiseptate spindle shaped macroconidia with blunt ends X400



TRICHOPHYTON

THE GENUS *Trichophyton* contains a large number of species which attack glabrous skin bearded areas hair and nails and causes a wide variety of lesions depending upon the species and the site of infection. Infected hairs show either chains of spores outside the shaft (ectothrix type) or parallel rows of spores within the shaft (endothrix type).

Cultural and Microscopic Characteristics

These fungi form cottony velvety finely granular to powdery or glabrous aerial mycelium and vary in color from white to brown violet to purple pink to red or yellow to orange.

The macroconidia (which cannot always be demonstrated in every species) are large clavate smooth thin walled multiseptate. The microconidia produced in abundance in some species are single celled and are globular clavate or pyriform in shape and may be borne singly along the sides of the hyphae (en thyrsé) or in grape like clusters (en grappe). Other structures which may be produced are spiraled hyphae nodular bodies racquet mycelium and chlamydospores. Typical of some species of *Trichophyton* is the formation of the knobbed hyphal structures called "fascic chandeliers" as their only identifying characters.

TRICHOPHYTON MENTAGROPHYTIS

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Downy type—white velvety to fluffy
aerial mycelium light buff to tan to
brown on reverse surface granular
type—white powdery to granular
aerial mycelium sometimes pinkish
light buff to yellow to reddish
brown pigment on reverse side

Cottony type—white fluffy aerial
mycelium light buff to tan on
reverse side

Corn meal agar plus
1% glucose

No pigment on undersurface

MICROSCOPIC

Sabouraud's glucose
agar wort or corn
meal agar

Microconidia microconidia spirals
nodular bodies

Cottony type Sabou-
raud's glucose agar
wort or corn meal
agar

Few or no microconidia

TRICHOPHYTON MENTAGROPHYTES

(Trichophyton gypsum)

THE SPECIES *Trichophyton mentagrophytes* includes many variants. This dermatophyte is the chief incitant of the inflammatory type of ringworm involving the feet (athlete's foot), hands, and glabrous skin particularly of the intertriginous areas. The fungus also attacks nails, bearded areas, and the scalp, causing the ectothrix type of hair.

Cultural and Microscopic Characteristics

The variants of this species on Sabouraud's glucose agar show marked differences in colonial appearance, the colonies having aerial mycelium ranging from cottony to powdery, of white to cream to tan or pinkish color with cream to tan to reddish brown pigment on the reverse surface.

Microscopically, there are found in the downy and granular types numerous clavate to pyriform microconidia borne singly along the sides of the hyphae (en thyrses) and in clusters (en grappe). Typical long clavate thin-walled multiseptate macroconidia with constriction at septa. Characteristic spiraled hyphae and nodular bodies may also be present. In some of the variants, particularly the fluffy or cottony forms, no identifying structures or only a few microconidia may be found.

TRICHOPHYTON MENTAGROPHYTES

Figure 4

Growth on Sabouraud's glucose agar after two weeks

A Cottony type

B Powdery to granular type

Microscopic structures—powdery type

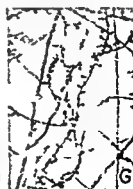
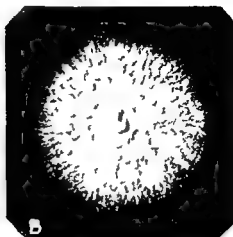
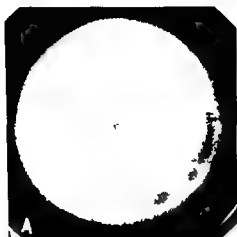
C Stained slide culture preparation showing microconidia in clusters
X 600

D Typical multiseptate clavate macroconidia X 400

E Culture mount from corn meal agar showing nodular bodies X 400

F Spiraled hyphae X 600

G Microconidia along sides of hyphae X 400



TRICHOPHYTON RUBRUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Cottony mycelium becoming velvety
to powdery with central umbo
reddish purple pigment on
reverse side

Corn meal plus
1% glucose

Wine red pigment on undersurface

MICROSCOPIC

Blood agar base
(Difco)

Microconidia microconidia

TRICHOPHYTON RUBRUM

(Trichophyton purpureum)

THE SPECIES *Trichophyton rubrum* is the commonest incitant of chronic dermatophytosis of the hands and feet nails and glabrous skin the fungus may also involve the bearded areas including the hair follicles

Cultural and Microscopic Characteristics

The colony on Sabouraud's glucose agar at an early stage is white fluffy and hemispheric later becoming velvety to powdery with a central umbo and sometimes showing radial folds on the undersurface there is a characteristic reddish purple pigment

Microscopically long slender thin walled multiseptate macroconidia (fuseaux) with parallel sides and large numbers of pyriform microconidia borne from sides of hyphae are found For demonstration of the macroconidia a special medium is required

TRICHOPHYTON RUBRUM

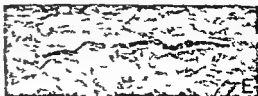
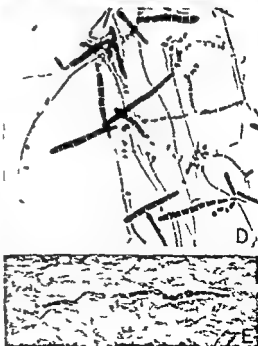
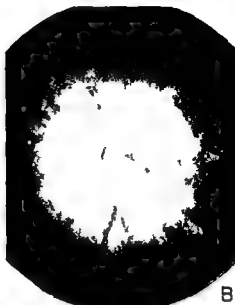
Figure 5

A Colony on Sabouraud's glucose agar after eight days

B Colony after three weeks

C and D Blood agar base slide culture preparations stained with lactophenol cotton blue showing microconidia along sides of hyphae and long, multiseptate microconidia X400

E Direct preparation from skin of patient with ringworm X400



TRICHOPHYTON TONSURANS

Media for Development of Significant Characters

MACROSCOPIC

Sibouraud's glucose agar	Heaped irregularly folded usually finely powdery aerial mycelium creamy white to tan to yellow or shades of rose on reverse dark brown pigment
-----------------------------	------------------------------------------------------------------------------------------------------------------------------------------------------------

MICROSCOPIC

Sibouraud's glucose agar	Microconidia irregularly branched hyphae many chlamydospores
Polished rice grains	Microconidia microconidia

TRICHOPHYTON TONSURANS

(Trichophyton crateriforme)

THE SPECIES *Trichophyton tonsurans* attacks hair, glabrous skin and nails. The hairs show the endothrix type of infection.

Cultural and Microscopic Characteristics

The culture is comparatively slow growing on Sabouraud's glucose agar. The colony is heaped and folded, sometimes showing a central crater or a central prominence with a surface of compact velvety or powdery aerial mycelium of creamy white to tan yellow or shades of rose; the pigment on the undersurface is dark brown.

Microscopically, there are large numbers of clavate or elongated microconidia attached singly to the sides of the hyphae or in loose clusters, and few thin-walled club-shaped macroconidia. Long, thick and irregularly branched hyphae and many chlamydospores.

TRICHOPHYTON TONSURANS

Figure 6

- A Colony on Sabouraud's glucose agar after five weeks
- B Sabouraud's glucose agar slide culture preparation stained with lactophenol cotton blue showing microconidia borne laterally on hyphae and in clusters $\times 400$
- C Growth from polished rice grains showing masses of microconidia $\times 600$
- D Growth from polished rice grains showing thin walled clavate microconidia $\times 400$



TRICHOPHYTON VERRUCOSUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Blood agar base
plus thiamine

Glabrous heaped up cerebriform
colony or disc shaped with white
velvety mycelium or a much
wrinkled glabrous colony of
bright yellow color

MICROSCOPIC

Blood agar base plus
thiamine

Polished rice grains

Irregularly branched hyphae
microconidia microconidia
chlamydospores

Microconidia microconidia
chlamydospores

TRICHOPHYTON VERRUCOSUM

(*Trichophyton fauiforme*)

THE SPECIES *Trichophyton verrucosum* includes three varieties *album* *discoides* *ochraceum*. This microorganism causes a suppurative type of ringworm involving the deeper layers of the skin and the hair follicles. Infected hairs show large spores arranged in chains forming a sheath on the outside of the hairs (ectothrix type) and mycelial elements within the hair shaft. Human infection is acquired directly or indirectly through contact with cattle having ringworm lesions. The fungus may be isolated on Sabouraud's glucose agar but usually an enriched medium is required.

Cultural and Microscopic Characteristics

The three varieties of *T. verrucosum* differ markedly in colonial appearance varying from a glabrous heaped up cerebriform slightly yellowish colony (*T. verrucosum* var. *album*) to a flat disc shaped colony covered with a short white aerial mycelium (*T. verrucosum* var. *discoides*) or a much wrinkled glabrous flat colony of a bright yellow color (*T. verrucosum* var. *ochraceum*).

Microscopically, there will be seen long multiseptate macroconidia with pointed or bulbous distal ends, elongated pear shaped microconidia borne sessile along the sides of the hyphae or in terminal clusters, irregularly branched hyphae with vast numbers of intercalary and terminal chlamydospores. Special media are essential for demonstration of macroconidia.

TRICHOPHYTON FERRUCOSUM

Figure 7

Colonies on blood agar base plus threonine

A *T. ferrucosum* var. *discoides* after two weeks

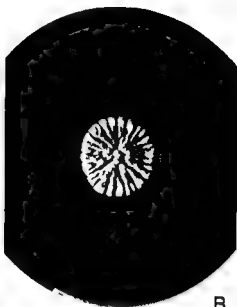
B *T. ferrucosum* var. *album* after three weeks

C *T. ferrucosum* var. *ochraceum* after four weeks

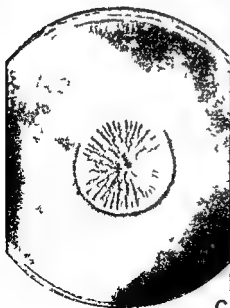
D Slide culture preparation stained with lactophenol cotton blue showing microconidia, clusters of microconidia and chains of chlamydospores $\times 400$



A



B



C



D

TRICHOPHYTON SCHOENII FIMI

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Glabrous, heaped and folded
sometimes showing a white
powdery surface

MICROSCOPIC

Sabouraud's glucose
agar

Fine cylindrical chlamydospores

TRICHOPHYTON SCHOENLEINI

(Achorion schoenleini)

THE FUNGUS *Trichophyton schoenleini* is the chief agent of favus, a chronic fungus infection of the scalp and sometimes of the glabrous skin and nails. The disease is rare in the United States. In infected hairs large endothrix spores in chains are found together with mycelial elements and characteristic air bubbles. The species can be isolated on Sabouraud's glucose medium but may be difficult to obtain in pure form due to bacterial contamination of the inoculum.

Cultural and Microscopic Characteristics

The colony on Sabouraud's glucose agar is slow growing, glabrous, heaped and folded, the surface later becoming covered with short white aerial mycelium. A characteristic of the colony is the cracking of the medium.

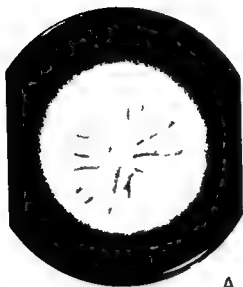
Microscopically the identifying structures are the favic chandeliers.

TRICHOPHYTON SCHOFNIINI

Figure 8

A Colony on Sabouraud's glucose agar after four weeks

B Sabouraud's glucose agar slide culture preparation showing "frie chandeliers" X400



A



B

TRICHOPHYTON VIOLEACEUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Glabrous heaped and folded deep
violet in color sometimes showing
fine white velvety mycelium
on surface

MICROSCOPIC

Sabouraud's glucose
agar

No identifying structures

TRICHOPHYTON VIOACEUM

THE SPECIES *Trichophyton violaceum* attacks the hair of the scalp and bearded areas the skin and nails. The infection is found chiefly in immigrants in the United States and causes one of the most refractory types of ringworm of the scalp of all the trichophyta. The hairs show the endothrix type of infection the large spores being arranged in rows within the hair shaft. The microorganism can be isolated on Sabouraud's glucose agar but growth may not be recognizable for three to four weeks.

Cultural and Microscopic Characteristics

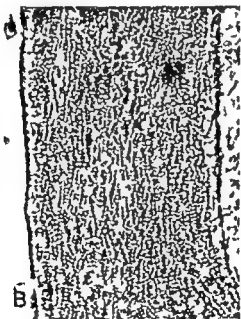
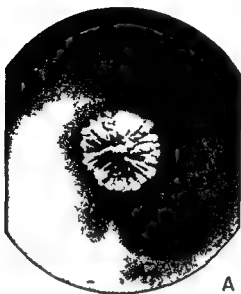
The colony on Sabouraud's glucose medium is moist glabrous heaped and folded and shows a deep violet color the surface at times becoming covered with a fine white short aerial mycelium.

Microscopically no identifying structures are found.

TRICHOPHYTON VIOLESCUM

Figure 11

- A Colony on Sabouraud's glucose agar after four weeks
- B Hair from ringworm of scalp showing endothrix spores X 600



EPIDERMOPHYTON FLOCCOSUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Velvety or felt like surface with or
without radial grooves greenish
yellow in color

MICROSCOPIC

Sabouraud's glucose
agar
Corn meal

Macroconidia chlamydospores

EPIDERMOPHYTON

THE GENUS *Epidermophyton* consists of 1 single species *E. floccosum* (*E. inguinale* *E. cruris*). This dermatophyte attacks the skin particularly of the groin and the nails. The hair is not affected.

Cultural and Microscopic Characteristics

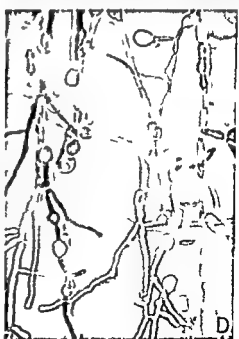
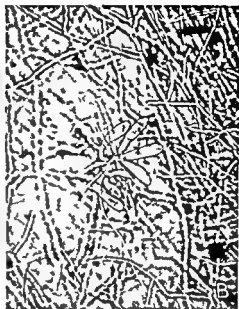
The colony on Sabouraud's glucose agar is moderately fast growing. The surface is velvety or felt like, often with radial furrows and an irregular folded center. or the surface may be smooth. the color is greenish yellow. A characteristic of this culture is the appearance on its surface of small tufts of white fluffy aerial mycelium.

Microscopically there will be seen the distinctive clavate macroconidia with blunt ends. they are smooth thin walled and multiseptate and are frequently borne in banana like clusters. numerous intercalary and terminal chlamydospores are also characteristic. no microconidia are produced.

EPIDERMOPHYTON FLOCCOSUM

Figure 10

- A Colony on Sabouraud's glucose agar after four weeks
- B Cluster of multiseptate club shaped macroconidia on Sabouraud's glucose agar $\times 400$
- C Numerous typical club shaped macroconidia $\times 400$
- D Chlamydospores $\times 600$



Deep-Seated Mycoses

(Subcutaneous Systemic)

ALMOST ALL THE deep seated mycoses have been encountered throughout the North American continent although the incitants are not necessarily endemic in all the areas where the diseases have been found. Shifting populations and travel in endemic areas play a large part in the appearance of some of these diseases in areas where they are totally unsuspected. As a consequence these infections frequently remain undiagnosed or are incorrectly diagnosed.

These diseases which may be fatal or relatively benign are localized or systemic and are usually chronic but may be acute or subacute. They are caused by a wide variety of fungi.

These fungi attack the internal organs of the body, bone, meninges, subcutaneous tissue, skin and mucous membranes. A definitive diagnosis cannot be established without the aid of the laboratory, since demonstration of the fungus in pathologic specimens either by microscopic examination or cultural isolation is essential.

A number of the most important fungi are described in the following pages under the specific diseases which they incite.

ACTINOMYCES BOVIS*Media for Development of Significant Characters***MACROSCOPIC**

Beef heart infusion
agar with blood
(anaerobic 7 to 10
days)

Colonies—white fused rough dry
1 mm or less in diameter with an
irregular surface and lobate edges

Beef infusion agar
with 1% glucose in
test tube* (aerobic
incubation)

No growth for 5 to 15 mm below
surface at upper level of growth
definite band from 2 to 7 mm in
width consisting of minute col-
onies below this many discrete
colonies of variable size and of
irregular shape

Beef infusion broth
with 1% glucose
(anaerobic 7 to 10
days)

Good growth clear supernatant
floccular or granular sediment tiny
colonies up sides of tube

MICROSCOPIC

Beef heart infusion
agar with blood
(anaerobic)

Crim positive branching filaments
long and short rods coccoid
forms

Beef infusion broth
with 1% glucose
(anaerobic 7 to 10
days)

Crim positive branching filaments
pleomorphic rods coccoid bodies

ACTINOMYCES BOVIS

Figure 11

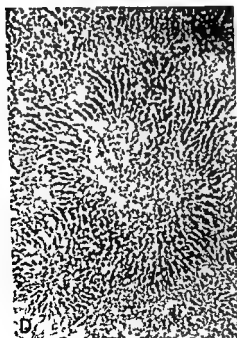
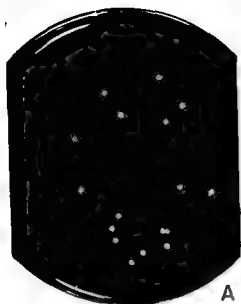
A Colonies on blood agar after two weeks incubation under anaerobic conditions

B Stained film preparation of culture from blood agar $\times 780$

C Stained film preparation of pus from abscess in experimentally infected hamster $\times 600$

D Unstained granules with clubs in pus from encapsulated abscess in experimentally infected hamster $\times 600$

E Section of encapsulated abscess showing granules with clubs and mass of filaments H & E $\times 900$



NOCARDIA ASTEROIDES*Media for Development of Significant Characters***MACROSCOPIC**

Sabouraud's glucose
agar slants
(22-25 or 37 C)

Gelatin

Milk containing
bromocresol purple
or litmus as indi-
cator

Raised wrinkled granular growth
ochre to yellow to orange in color
sometimes becoming covered with
chalky white aerial mycelium

No liquefaction after 2 to 4 weeks
at 22-25 or 37 C

Surface pellicle no coagulation
no peptonization alkaline reaction
after 4 weeks at 22-25 or 37 C

MICROSCOPIC

Sabouraud's glucose
agar

Milk with indicator
(3 weeks at 22-25
C)

Gram positive branching filaments
fragmenting filaments bacillary
and coccoid forms

Acid fast fragmenting filaments
bacillary and coccoid forms

NOCARDIOSIS

NOCARDIOSIS is a disease similar to or even clinically indistinguishable from actinomycosis. The etiologic agent may be one of several species of the genus *Nocardia*. *N. asteroides* is the most commonly encountered species in the United States and is therefore described below.

Specimens for Examination

Pus, sputum, cerebrospinal fluid, biopsy specimens or post mortem material may be submitted.

Cultural and Microscopic Characteristics

N. asteroides is an aerobic, Gram positive, acid fast, filamentous, branching microorganism which breaks up readily into short rods or coccoid forms. In sputum, pus or other exudates it occurs in these forms. In abscesses it may also be found in the form of granules with or without peripheral clubs. The acid fast property of this species is relatively feeble as compared with the tubercle bacillus. In culture this property can best be demonstrated by growing the microorganism in skim milk.

The microorganism is easily isolated on Sabouraud's glucose agar at 22-25° or 37° C. The colony on this medium is slow growing, dry, wrinkled and granular with a slightly heaped center and an irregular, lobulate margin. The color is at first whitish, changing to ochre and later to orange, sometimes becoming covered with a chalky white aerial mycelium. In film preparations stained by Gram's method there will be seen long and short Gram positive branching filaments, fragmenting filaments, short bacillary and coccoid forms.

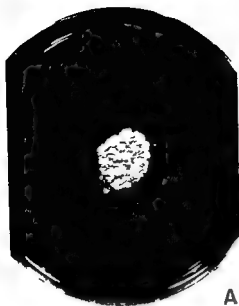
Pathogenicity for Animals

The microorganism has a high degree of pathogenicity for the guinea pig.

NOCARDIA ASTEROIDES

Figure 12

- A Colony on Sabouraud's glucose agar after four weeks
- B Stained film preparation of culture in milk showing acid fast filaments and rods $\times 1500$
- C Stained film preparation of pus from omentum of experimentally infected guinea pig showing fragmenting branching filaments $\times 600$
- D Section of guinea pig omentum showing abscess with central granule H & E $\times 600$



A



B



C



D

BLASTOMYCIS DERMATITIDIS*Media for Development of Significant Characters***MACROSCOPIC**

Blood agar (37 °C)	Cream to tan soft wrinkled and waxy in appearance
Sabouraud's glucose agar (22-25 °C)	White fluffy aerial mycelium becoming cream to tan

MICROSCOPIC

Blood agar (37 °C)	Large round single budding thick walled cells
Sabouraud's glucose agar (22-25 °C)	Round to pyriform microconidia borne laterally on hyphae

BLASTOMYCOSIS

North American Blastomycosis (Gilchrist Disease)

THIS IS A CHRONIC DISEASE which may be localized or systemic. It is often localized in the skin forming granulomatous lesions with a verrucous surface and a raised smooth border sloping sharply to the normal skin. The systemic form usually follows a primary pulmonary infection and may spread by the blood stream affecting any other organ or tissue of the body. The etiologic agent is *Blastomyces dermatitidis*.

Specimens for Examination

Bits of tissue from skin lesions pus from subcutaneous abscesses sputum bone blood or cerebrospinal fluid may be submitted.

Cultural and Microscopic Characteristics

B. dermatitidis exhibits dimorphism i.e. it grows in one form in tissue and in another form on culture media. In pathologic material it appears as a large spherical thick walled budding cell of variable size 8 to 20 μ in diameter. on Sabouraud's glucose agar at room temperature it appears in the mycelial form.

On blood agar medium at 37° C the growth is soft wrinkled and waxy in appearance cream to tan in color while on Sabouraud's glucose medium at room temperature the growth consists of white downy or fluffy aerial mycelium later becoming cream to tan to brown.

Microscopically the culture on blood agar medium at 37° C appears in the same form as in tissue while on Sabouraud's glucose medium the growth consists of branched septate hyphae on which are borne small round to pear shaped conidia sessile or on short lateral branches.

A culture thought to be *B. dermatitidis* should not be identified as such without demonstration of its dimorphic nature.

Pathogenicity for Animals

The microorganism is pathogenic for the young male hamster and to a lesser degree for the white mouse guinea pig and rabbit.

BLASTOMYCES DERMATITIDIS

Figure 13

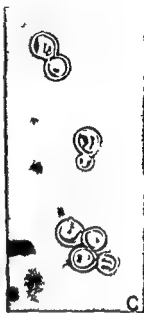
- A Colony on Sabouraud's glucose agar after three weeks at room temperature
- B Culture after 72 hours on blood agar at 37° C.
- C Culture mount of growth on blood agar X 800
- D Sabouraud's glucose agar slide culture preparation showing small round to pear shaped conidia X 400
- E Large spherical thick walled budding cells of variable size in lung tissue of man stained by Hotchkiss McManus technique X 400



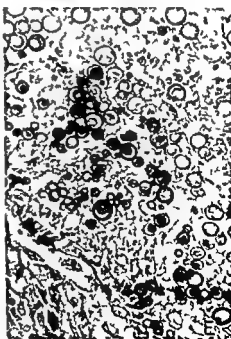
A



B



C



BLASTOMYCOSIS

South American Blastomycosis (Lutz Splendore Alencar Disease)

This is a chronic granulomatous disease of many manifestations depending upon the tissue involved. It may remain localized or become disseminated. Initial lesions which are commonest on the mucous membrane of the mouth and pharynx may also be localized on the nasal or anorectal membrane, the skin and in the lungs. Frequently the liver is infected from the lungs. Dissemination takes place by the hematogenous and lymphatic routes. The etiologic agent is *Paracoccidioides brasiliensis* (*Blastomyces brasiliensis*).

Specimens for Examination

Bits of tissue from cutaneous or mucosal lesions, pus from lymph nodes or from cutaneous abscesses, sputum, bone, blood, biopsy material or tissues from post mortem examinations may be submitted.

Cultural and Microscopic Characteristics

P. brasiliensis is a dimorphic fungus. In pathologic materials the microorganism appears in the form of single and multiple budding, thick-walled yeastlike cells 10 to 60 μ in diameter and on artificial medium at room temperature in a mold like or mycelial form.

On blood agar or chocolate agar at 37 C the colonies are white yeastlike and cerebriform. On Sabouraud's glucose agar at room temperature the colony is compact, irregularly folded and covered with a short matted white aerial mycelium becoming brownish in old cultures.

Microscopically the growth on blood agar at 37 C consists of many single and multiple budding cells identical with the tissue forms while on Sabouraud's glucose medium the growth at room temperature is composed of branched septate hyphae.

A mycelial culture thought to be *P. brasiliensis* should not be identified as such without conversion to the yeast phase and demonstration of multiple budding cells

Pathogenicity for Animals

The microorganism has been reported as pathogenic for the guinea pig rabbit hamster and white mouse

PARACOCCIDIOIDES BRASILIENSIS

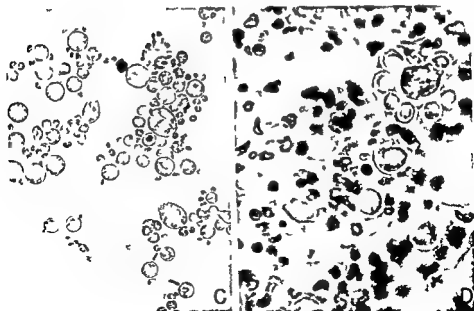
Figure 14

A Colony on Sabouraud's glucose agar after three weeks at room temperature

B Growth on blood agar after seventy two hours at 37 °C

C Culture mount from blood agar at 37 °C in lactophenol cotton blue X 600

D Large spherical thick walled single and multiple budding cells in human tissue H & E X 800 (Courtesy of Dr Margarita Silva Department of Dermatology College of Physicians and Surgeons Columbia University New York NY)



CANDIDA ALBICANS

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

Creamy pasty smooth

MICROSCOPIC

Sabouraud's glucose
agar

Round to oval budding cells

Corn meal agar
dispensed in Petri
platesMycelium blastospores
chlamydospores

MONILIASIS (CANDIDIASIS)

MONILIASIS is a disease of many manifestations. It may involve the skin particularly the intertriginous areas, nails (onychia) and tissue around the nails (paronychia), the mucous membranes (mouth, pharynx, vagina), the perianal area, broncho-pulmonary system or even the blood stream or meninges. The chief etiologic agent is *Candida albicans*, other species which may cause infection are *C. tropicalis*, *C. guilliermondii*, *C. parapsilosis*, *C. pseudotropicalis* and *C. krusei*. *C. albicans*, because of its greater importance, is described in more detail. A table with the differential characteristics of the six species is included.

Specimens for Examination

Skin and nail scrapings, exudates from mucous membranes, sputum, feces, blood or cerebrospinal fluid may be required for examination.

Cultural and Microscopic Characteristics

C. albicans is a budding yeastlike microorganism which forms a true mycelium as well as pseudomycelium. It appears in pathologic materials as budding cells and mycelial elements.

It is easily isolated on Sabouraud's glucose agar. The colony is cream colored, pasty and smooth and has a yeastlike odor. Microscopically there are found budding cells of varying shape which are of little aid in the specific identification of the microorganism.

For identification the demonstration of the large, round, thick-walled chlamydospore characteristic of *C. albicans* is essential. This may be accomplished by growing the microorganism on corn meal agar. The corn meal agar dispensed in Petri plates should be inoculated with the primary culture by cutting through the agar along the line of streak and incubating at room temperature for several days. Along the line of streaks *C. albicans* develops mycelium on which is produced grape-like clusters of blastospores and the round, thick-walled chlamydospores characteristic of this species.

Pathogenicity for Animals

This fungus is pathogenic for mice and rabbits.

CANDIDA ALBICANS

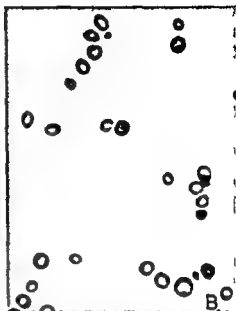
Figure 15

A Colony on Sabouraud's glucose agar after two weeks

B Culture mount from Sabouraud's agar $\times 600$

C Culture mount from corn meal agar showing clusters of blastospores and large round thick walled cells (chlamydospores) along hyphae $\times 400$

D Budding cells and mycelial elements in skin scrapings stained by Hotchkiss McManus technique $\times 400$ (Preparation courtesy of Mr J. Dennis Pollack of the Department of Dermatology, College of Physicians and Surgeons, Columbia University)



COCCIDIODES IMMITIS

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

White cottony aerial mycelium
becoming tan with age having
a central zone of flat sparse
mycelium

MICROSCOPIC

Sabouraud's glucose
agar

Coarse branching hyphae and chains
of thick walled chlamydospores

COCCIDIOIDOMYCOSIS

COCCIDIOIDOMYCOSIS caused by *Coccidioides immitis* occurs in two forms—an acute self limiting respiratory infection (California disease valley fever) and a chronic sometimes fatal disease (coccidioidal granuloma) involving the cutaneous subcutaneous visceral and bony tissues

Specimens for Examination

Exudates from cutaneous lesions pus from abscesses sputum cerebrospinal fluid blood biopsy material or tissues from post mortem examination may be submitted

Cultural and Microscopic Characteristics

C. immitis is dimorphic In the parasitic phase it appears as thick walled endosporeulating spherules 5 to 50 μ in diameter in different stages of development In culture at 37 C or at room temperature it occurs only in the mycelial form

The microorganism is easily isolated on Sabouraud's glucose medium or on Mycosel agar if the specimen is grossly contaminated The colony is moderately fast growing flat moist and membranous later developing a coarse white to tan to brown cottony aerial mycelium with central zone of flat sparse mycelium

Microscopically the culture shows coarse branching hyphae some broken up into thick walled spores chlamydospores usually in chains

Great precaution should be exercised when handling these cultures since the chlamydospores are easily dislodged and carried through the air Cultures on medium in Petri plates should not be employed Petroff flasks (22 x 95 mm) or tubes should be used instead

Pathogenicity for Animals

This fungus is pathogenic for white mice guinea pigs and rabbits The guinea pig injected intratesticularly with a suspension of the chlamydospores develops a severe orchitis within seven to ten days From such lesions pus may be aspirated and the typical endosporeulating spherules demonstrated by direct microscopic examination

A culture thought to be *C. immitis* should not be identified as such without demonstration of transformation in a susceptible animal of the chlamydospores to the characteristic parasitic growth phase (thick walled endosporeulating spherules)

COCCIDIOIDES IMMITIS

Figure 17

- A Colony on Sabouraud's glucose agar after five weeks
- B Culture mount showing chains of chlamydospores $\times 400$
- C Spore $\times 800$
- D Section from testicle of experimentally infected guinea pig, showing endosporulating spherules H & E $\times 400$
- E Spherule $\times 800$



A



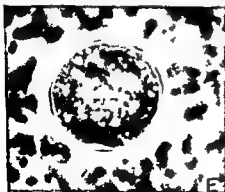
B



D



C



E

CRYPTOCOCCUS NEOFORMANS

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar
(22-25 and 37
C)

Cream to tan in color usually
mucoid and glossy occasionally
dry and dull

MICROSCOPIC

Sabouraud's glucose
agar

Round budding cells of varying
size surrounded by capsules of
different diameters

CRYPTOCOCCOSIS

CRYPTOCOCCOSIS is a subacute or chronic infection chiefly of the central nervous system but may involve almost any part of the body. The etiologic agent is *Cryptococcus neoformans* (*Torula histolytica*)

Specimens for Examination

Cerebrospinal fluid scrapings from superficial skin lesions pus from subcutaneous abscesses sputum bone lymph nodes blood bone marrow or specimens collected at post mortem may be submitted

Cultural and Microscopic Characteristics

Cryptococcus neoformans is a budding yeastlike microorganism. In pathologic material and in culture it appears as a round occasionally oval budding cell of variable size 4 to 15 μ in diameter surrounded by a refractile mucinous capsule. The capsule varies in width sometimes being twice the diameter of the cell and can be demonstrated by mounting a loopful of the infected material or culture in a drop of India ink.

The fungus is easily isolated on Sabouraud's glucose medium. The colony is mucoid and slimy and is cream to brownish in color.

The pathogenic strains grow readily at 22 25 and 37 C and do not assimilate KNO_3 while the nonpathogenic or avirulent strains grow poorly if at all at 37 C and assimilate KNO_3 .

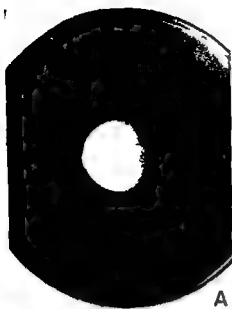
Pathogenicity for Animals

The microorganism is virulent for white mice and rats. For proof of the virulence of a given strain of *C. neoformans* mice should be injected intravenously or intracerebrally and following the death of the animal budding cells with large capsules demonstrated in the brain.

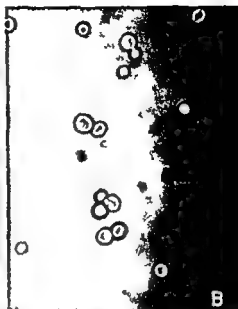
CRYPTOCOCCUS NEOFORMANS

Figure 18

- A Colony on Sabouraud's glucose agar after 10 days
- B Culture mount from Sabouraud's agar in drop of lactophenol
 \ 150
- C India ink preparation of culture from Sabouraud's agar \ 100
- D Section of spleen from white rat showing budding cells surrounded by capsules H & E \ 525



A



B



C



D

GEOTRICHUM CANDIDUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose agar	Flat membranous soft veistlike consistency white to cream in color
Honey broth	White pellicle

MICROSCOPIC

Sabouraud's glucose agar	Chains of rectangular to round arthrospores
Corn meal agar	

GEOTRICHOSIS

GEOTRICHOSIS is a comparatively rare and mild disease. Lesions may occur in the mucous membranes of the mouth and intestinal tract in the bronchi and lungs. The etiologic agent is *Geotrichum candidum*.

This microorganism may be present on the skin in the mouth and in the feces of normal persons or is a secondary invader in pulmonary disease particularly tuberculosis. A diagnosis of bronchial or pulmonary geotrichosis should therefore not be made without repeated demonstration of the presence of the fungus in the sputum by both microscopic and cultural examination and the elimination of other bronchopulmonary diseases.

Specimens for Examination

Exudates from lesions in the mouth, sputum or bloody stools may be submitted.

Cultural and Microscopic Characteristics

G. candidum appears in pathologic material as large rectangular to oval cells (arthrospores) and round thick walled non budding cells. It can be easily isolated on Sabouraud's glucose agar.

The colony on the Sabouraud's medium is flat, membranous, soft of yeastlike consistency, white to cream in color and closely adherent to the medium. A differential feature is the formation of a white pellicle in liquid medium.

Microscopically it reveals broad septate mycelium and chains of rectangular to round arthrospores formed by fragmentation of the hyphae.

Pathogenicity for Animals

Pathogenicity for laboratory animals has not been demonstrated.

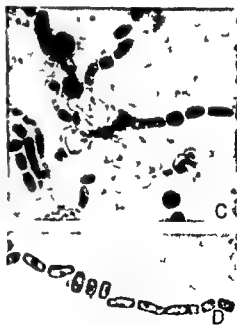
GIOTRICHUM CANDIDUM

Figure 19

- A Colony on Sabouraud's glucose agar after three weeks
- B Slide culture preparation on corn meal agar stained with lacto-phenol cotton blue showing arthrospore formation $\times 400$
- C and D Same showing rectangular and round arthrospores $\times 500$



A



D

HISTOPLASMA CAPSULATUM

Media for Development of Significant Characters

MACROSCOPIC

Blood agar (37 C)	Moist white yeastlike growth
Sabourauds glucose agar (22-25 C)	White fluffy aerial mycelium gradually becoming tan to brown

MICROSCOPIC

Blood agar (37 C)	Small oval budding cells
Potato glucose agar (22-25 C)	Round to pyriform microconidia borne laterally on hyphae and large thick walled tuberculate chlamydospores (microconidia)

HISTOPLASMOSIS

HISTOPLASMOSIS is a localized or generalized infection essentially of the reticuloendothelial system and may be acute subacute or chronic involving almost any organ of the body. The incitant is found chiefly as an intracellular parasite of the mononuclear cells in the peripheral blood, sternal bone marrow, lymph nodes or spleen. The etiologic agent is *Histoplasma capsulatum*.

Specimens for Examination

Tissue obtained by biopsy of lesions from skin, mucous membranes and lymph nodes, sputum, gastric washings or tissue from post mortem examination, including the brain, may be submitted.

Cultural and Microscopic Characteristics

H. capsulatum is dimorphic. In parasitized tissue it is found as small oval yeastlike cells 2.5 μ in the longer diameter within the large monocytes. On Sabouraud's glucose agar at room temperature it appears in the mycelial form.

Colonies on blood agar at 37° C are small, white, yeastlike. On Sabouraud's glucose agar at room temperature they are downy or cottony and white to cream, later becoming tan to brown.

Microscopically the culture on blood agar at 37° C occurs in the same form as in tissue, while on Sabouraud's glucose agar it consists of septate hyphae on which are borne two types of spores: (1) small, smooth-walled, round to pyriform conidia sessile on the sides of the hyphae or on very short lateral branches; (2) characteristic large, thick-walled, spiny or tuberculate chlamydospores (macroconidia).

For the identification of a culture thought to be *H. capsulatum*, the demonstration of the dimorphic nature of the fungus, as well as the presence of the tuberculate chlamydospores, is essential.

Pathogenicity for Animals

This fungus is pathogenic for mice, young hamsters, guinea pigs and rabbits.

HISTOPLASMA CAPSULATUM

Figure 20

A Colony on Sabouraud's glucose agar after three weeks at room temperature

B Growth on blood agar after 72 hours at 37° C

C Growth from blood agar at 37° C in lactophenol $\times 100$

D Culture from potato glucose agar showing small smooth walled round to pyriform conidia and tuberculate chlamydospores $\times 100$

E Large thick walled tuberculate chlamydospores stained with lactophenol cotton blue $\times 1000$

F Granulomatous lesion from ear of experimentally infected rabbit showing multinucleated giant cell filled with *H. capsulatum* H & E $\times 1500$



A



B



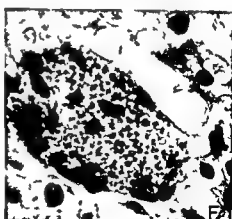
C



D



E



F

ASPERGILLOSIS

ASPERGILLOSIS is an inflammatory granulomatous infection. The skin, nails, external auditory canal, paranasal sinuses, eye, bronchi, bone, meninges, and brain may be involved. Several species of *Aspergillus* have been incriminated as agents of the disease. *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. glaucus*, and *A. niger*. *A. fumigatus* is by far the most frequently encountered. These fungi are ubiquitous in nature and are commonly saprophytic. For reasons not understood, they may become pathogenic and incite infection.

Pulmonary aspergillosis as a primary infection is well recognized and occurs most often in agricultural workers, squib feeders, fur cleaners, and other persons in contact with fungus contaminated grain. Its occurrence as a complication of bacterial infection of the lungs has become increasingly important with the widespread use of antibiotics, steroids, and folate acid antagonists. The portal of entry is usually the respiratory tract from which the infection may become widely disseminated and terminate fatally.

Specimens for Examination

Sputum, skin material from ear and biopsy, and post mortem specimens may be submitted.

The disease is difficult to diagnose with certainty since the causative agent usually *A. fumigatus* is extremely widespread in nature and may appear in culture media or in pathologic specimens as a contaminant. The mere isolation or even the identification of the fungus is not sufficient. Demonstration of the fungus in tissue by direct microscopic examination is essential.

Cultural and Microscopic Characteristics

A. fumigatus is a filamentous fungus. In sputum or tissue it appears as short, branching, hyphal fragments, often with many small (2 to 4 μ) round green spores scattered throughout the field. Occasionally the entire fruiting structure may be present. The fungus is easily isolated on Sabouraud's glucose agar at room

temperature or at 37° C. Where there is marked bacterial contamination streptomycin and penicillin should be added to this medium. Medium to which cycloheximide (Actidione) has been added should not be used. The colony is first growing white at first soon becoming green or dark green flat and velvety. The fungus grows well at 45° C. or higher.

Microscopically the identifying structure of this species as well as all species of *Aspergillus* is the conidiophore with the large terminal vesicle bearing many sterigmata (phialides) from which chains of spores are produced. The conidiophore of *A. fumigatus* is smooth walled and the conidial head columnar and compact with sterigmata in one series. conidia are globose green and echinulate.

Pathogenicity for Animals

The fungus is pathogenic to a high degree for the rabbit and to a lesser extent for the guinea pig.

ASPERGILLUS FUMIGATUS

Figure 21

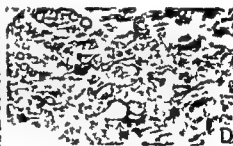
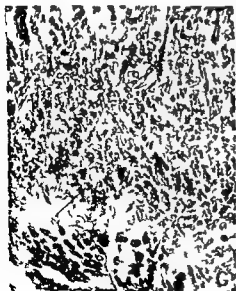
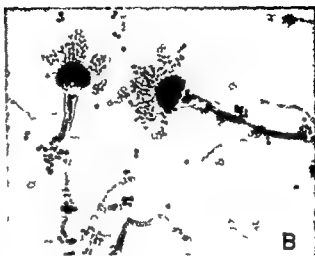
A Colony on Sabouraud's glucose agar seven days at room temperature

B Slide culture preparation on corn meal agar showing conidial heads \ 600

C Section from human lung showing septate hyphae H & E \ 400

D Same section showing conidial head (arrow) \ 600 (Courtesy of Dr. J. Lebowich, Stratton County Laboratory, Stratton Springs, N.Y.)

E Hyphal fragment of *A. fumigatus* in sputum (Reprinted with permission from Skinner, C. E., Limmons, C. W., Tsuchiya, H., M. Henriks: Yeasts, Molds, and Actinomycetes, 1947, John Wiley & Sons, Inc.)



SYSTEMIC MUCORMYCOSIS

SYSTEMIC MUCORMYCOSIS is a disease characterized by vascular invasion with growth of hyphae thrombosis infection and reactive inflammation of hemorrhagic exudative and minimal granulomatous character. The infection may be incited by any one of several species of three genera (*Mucor*, *Rhizopus* and *Absidia*) of the order *Mucorales* of the class *Phycomycetes*. The disease is being seen with increasing frequency, the pulmonary and cerebral tissues being most commonly attacked, often with a fatal outcome. These fungi may attack other tissues such as orbital, nasal and paranasal sinuses and intestine and produce relatively mild unrecognized infection. From such sites dissemination may occur with a rapidly fatal result.

These *Phycomycetes* are ubiquitous and usually saprophytic but under certain conditions apparently have the capacity to incite disease. Their spores become airborne and enter the nose sinuses and lungs by inhalation or the gastrointestinal tract on contaminated food.

There are several predisposing factors: acidotic diabetus melitus, leukemia and other debilitating diseases, the use of steroids, folic acid antagonists and prolonged antibiotic therapy is also believed to enhance susceptibility.

Specimens for Examination

Materials from sinuses, sputum, pus, cerebrospinal fluid and biopsy and post mortem tissues may be submitted.

The laboratory diagnosis of mucormycosis is not easy and should be made only under carefully controlled conditions since the etiologic agents are extremely widespread in nature and may appear as contaminants on media or in pathologic specimens. Demonstration of broad nonseptate hyphae by direct microscopic examination of pathologic specimens and isolation and identification of the fungus are essential for a definitive diagnosis.

Cultural and Microscopic Examination

The *Mucorales* are nonseptate filamentous fungi. In sputum

pus or tissue they appear as wide (limits of 4 to 50 μ) nonseptate branching hyphal fragments; occasionally the entire fruiting structure may be found. They are easily isolated at room temperature on Sabouraud's glucose agar with the addition of streptomycin and penicillin or at 37° C on blood medium with antibiotics. The colony grows rapidly, quickly filling a Petri plate or test tube with fluffy mycelium, first white, later dark gray.

Microscopically the hyphae are wide, colorless and coenocytic. The identifying structures are the single or branched sporangia, ophores which arise from any point on the hyphae or opposite rhizoids and bear large terminal globose spore-filled structures, the sporangia. (See pages 132-133.)

Pathogenicity for Animals

The *Mucorales* have only slight, if any, pathogenicity for the normal rabbit. Cerebral mucormycosis has been produced experimentally in rabbits with alloxan-induced diabetes by intranasal instillation of spore suspensions of these fungi.

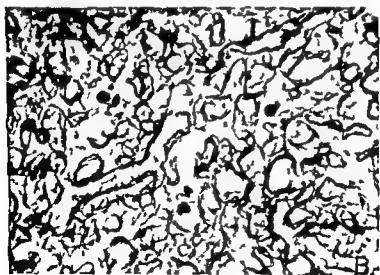
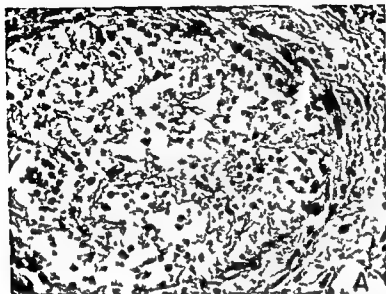
AGENT OF MUCORMYCOSIS

Figure 22

Section of human lung showing

A Wide nonseptate hyphae in pulmonary artery H & E $\times 300$

B Same in lumen $\times 700$



MONOSPORIUM APIOSPERMUM

Media for Development of Significant Characters

MACROSCOPIC

Sabouraud's glucose
agar

White cottony aerial mycelium
becoming gray and spreading
rapidly over entire surface of
media blackened undersurface

MICROSCOPIC

Corn meal agar

Light brown single ovoid conidia at
tips of long or short conidiophores
occasionally on the sides of hyphae

MADUROMYCOSIS

MADUROMYCOSIS is a chronic granulomatous disease usually involving only one of the lower extremities characterized by enlargement and deformity multiple draining sinuses and bone destruction. The material from discharging sinuses generally contains granules (grains) irregular shaped masses of various colors white yellow red or black. Among the incitants are species of *Madurella*, *Aspergillus*, *Cephalosporium*, *Allescheria* and *Monoascus*. *M. apiospermum* one of the chief incitants is described below.

Specimens for Examination

Discharges from fistulae material aspirated from deep cutaneous and subcutaneous nodules may be submitted.

Cultural and Microscopic Characteristics

M. apiospermum is a filamentous fungus. In pathologic material the fungus appears in the form of yellowish white granules lobulated structures composed of wide septate hyphae with numerous peripheral chlamydospores.

It is readily isolated on Sabouraud's glucose agar at room temperature. The colony on this medium is fast growing white cottony gradually becoming gray and has a grayish to black pigment on the undersurface.

Microscopically there will be seen wide septate hyphae and large round oval or clavate conidia borne singly at tips of short or long conidiophores.

M. apiospermum is the imperfect form of *A. boydii* also an etiologic agent of maduromycosis. *A. boydii* produces asexual spores identical with *M. apiospermum*. For its identification the demonstration of perithecia (ascocarps) large (20-150 μ) spherical brown to black structures is essential. (See Figure 22C.)

Pathogenicity for Animals

The pathogenicity of these species for small laboratory animals has not been established.

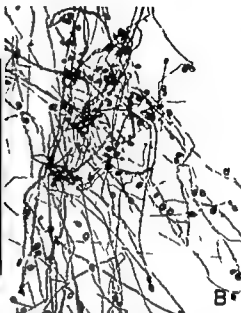
MONOSPORIUM APIOSPERMUM

Figure 23

- A Colony on Sabouraud's glucose agar after five weeks
- B Culture mount from corn meal agar stained with lactophenol cotton blue showing single terminal conidia on long and short conidiophores $\times 400$
- C Culture mount of *Allescheria boydii* from Sabouraud's glucose agar eight weeks showing young and mature ascocarps; note large ripe ascocarp discharging ascospores $\times 800$
- D Granule from experimentally infected hamster H & E $\times 900$



A



B



C



D

SPOROTRICHUM SCHENCKII

Media for Development of Significant Characters

MACROSCOPIC

Glucose cystine blood agar (37 C)	Soft yeastlike growth
Sibourauds glucose agar (22-25 C)	Leathery wrinkled and folded tan to brown to black

MICROSCOPIC

Glucose cystine blood agar (37 C)	Round oval fusiform budding cells
Corn meal agar (22-25 C)	Fine septate hyphae and pear shaped microconidia borne terminally in rosette like clusters on lateral branches or singly along the hyphae

SPOROTRICHOSIS

THIS DISEASE is primarily a chronic infection of the skin and subcutaneous tissues characterized by nodular lesions which spread along the regional lymphatics. Generalized infections do occur but are rare in the United States. The localized lesions are most frequently found on the extremities particularly the hands. The etiologic agent is *Sporotrichum schenckii* (*S. beurmanni*).

Specimens for Examination

Pus from subcutaneous nodules or from ulcerating lesions may be submitted.

Cultural and Microscopic Characteristics

S. schenckii is dimorphic. In pus and necrotic tissue from human infection this microorganism appears as single-celled cigar shaped bodies usually within the polymorphonuclear leukocytes (these structures are extremely difficult to demonstrate in pus or tissue sections from human lesions). On Sabouraud's glucose agar at room temperature it grows in the mycelial form.

On glucose cystine blood agar at 37° C. the growth is creamy white soft and yeastlike. On Sabouraud's glucose medium at room temperature the microorganism forms a mycelial colony at first white and soft later becoming tan to brown to black and leathery in texture and having a convoluted center from which extend radial grooves terminating in a flat border. The appearance of the colony on this medium is diagnostic.

Microscopically the culture on the blood medium at 37° C. is found in the same form as in tissue while on Sabouraud's glucose medium the culture shows a fine branching septate mycelium and many small pear shaped conidia. The conidia are borne terminally in rosette like clusters on lateral branches or are attached singly along the hyphae.

Pathogenicity for Animals

The fungus is infectious for white male rats and mice and

SPOROTRICHUM SCHENCKII

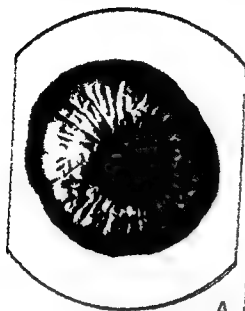
Figure 24

A Dark brown to black colony on Sabouraud's glucose agar after three weeks at room temperature

B Culture mount of growth from glucose cystine blood agar at 37 C X400

C Culture mount from corn meal showing fine branching hyphae and pear shaped conidia borne in rosette like clusters at tips of lateral branches (conidiophores) and singly along sides of hyphae X600

D Section from testicle of experimentally infected hamster showing Gram positive cigar shaped bodies Gram Weigert X600



A



B



C



D

AGENTS OF CHROMOBLASTOMYCOSIS

MACROSCOPIC

(On Sabouraud's glucose agar)

<i>Phialophora</i> <i>verrucosa</i>	Colonies slow growing dark green to black felt like velvet mycelium
<i>Cladosporium</i> <i>carrionii</i>	
<i>Fonsecaea</i> <i>pedrosoi</i>	
<i>F. compacta</i>	Extremely slow growing
<i>F. dermatitidis</i>	Black yeastlike at first gradually becoming filamentous at the periphery

MICROSCOPIC

(On corn meal or Czapek's agar)

<i>Phialophora</i> <i>verrucosa</i>	<i>Phialophora</i> type of sporulation only cup shaped conidiophores with spores that bud from the base of the cup
<i>Cladosporium</i> <i>carrionii</i>	<i>Cladosporium</i> type of sporulation only tree like branching conidiophores with conidia in abundance
<i>Fonsecaea</i> <i>pedrosoi</i>	3 types of sporulation single or combined in one head (a) <i>Cladosporium</i> (b) <i>Phialophora</i> and (c) pseudo- <i>Acrotheca</i> conidia borne laterally on short protuberances on conidiophore <i>F. compacta</i> differs from <i>F. pedrosoi</i> in its broad based conidia <i>F. dermatitidis</i> in its yeast like and <i>Fullularia</i> phases
<i>F. compacta</i>	
<i>F. dermatitidis</i>	

CHROMIOBLASTOMYCOSIS

CHROMIOBLASTOMYCOSIS caused by several species of dematiaceous fungi was defined by Dr A. L. Carrion (1947) as a chronic infectious apparently non contagious skin disease confined most frequently to one of the lower extremities and characterized clinically by the formation of nodular verrucous or tumor like lesions. More recently evidence of systemic disease has been reported. There are at least five species representing three genera which may cause this disease: *Phialophora verrucosa*, *Fonsecaea* (*Hormodendrum*) *pedrosoi*, *F. compacta*, *F. dermatitidis* and *Cladosporium carrionii*.

Specimens for Examination

Crusts or exudates from the verrucous lesions may be submitted.

Cultural and Microscopic Characteristics

These fungi are dimorphic. In tissue pus or caseous material they are indistinguishable. They appear as large round yellowish brown thick walled often septate bodies. On Sabouraud's glucose medium they are filamentous at 22-25° and 37° C.

The colonies on this medium are slow growing dark green to black felt like in appearance with the exception of *F. dermatitidis* and slightly embedded in the substrate. They cannot be differentiated by study of macroscopic characters alone due to their striking cultural similarity. *F. dermatitidis* is at first yeast like gradually becoming mycelial at the periphery.

Species identification is based upon the method of sporulation of which there are three types: *Phialophora* characterized by cup shaped conidiophores with spores which bud from the base of the cup; *Cladosporium* by tree like branching conidiophores

with conidia in chains. *Acrotheca* by swollen conidiophores with lateral protuberances from which are borne singly ovoid conidia. These three types of sporulation may occur simultaneously in a culture. *F. dermatitidis* in addition to the three types of sporulation also shows at a very early stage yeastlike and *Pullularia* phases.

P. verrucosa exhibits the *Phialophora* type of sporulation only.

F. pedrosoi, the most common incitant of chromoblastomycosis possesses a triple sporulating ability. All three types of sporulation occur simultaneously and any one may predominate.

F. compacta exhibits the three types of sporulation, sometimes all combined in a single spore head with the *Cladosporium* type predominating.

F. dermatitidis exhibits early yeastlike and *Pullularia* phases and later reveals in addition the three types of sporulation characteristic of the genus *Fonsecaea*.

C. carrionii shows the *Cladosporium* type of sporulation only. The pathogenic strains must be differentiated from saprophytic species of this genus on the basis of cultural characters. The pathogens grow at 22, 25 and 37° C and are slow growing whereas the saprophytic forms are fast growing and do not grow at 37° C.

Pathogenicity for Animals

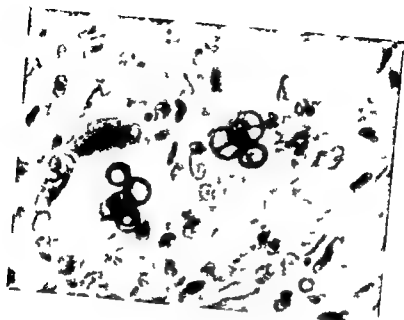
Rats and mice appear to be susceptible to these fungi injected by the intraperitoneal or intratesticular route.

Grateful acknowledgment is made to Dr. Margarita Silva for her assistance in the preparation of this section.

CHROMIOBLASTOMYCOSIS

Figure 25

Histopathologic section from lesion in chromoblastomycosis showing large septate bodies X800 (Courtesy of the late Dr Rhoda Benluram Department of Dermatology College of Physicians and Surgeons Columbia University)



**PHIALOPHORA VERRUCOSA
FONSICARIA PEDROSI
FONSICARIA DERMATITIDIS**

Figure 26

Phialophora verrucosa

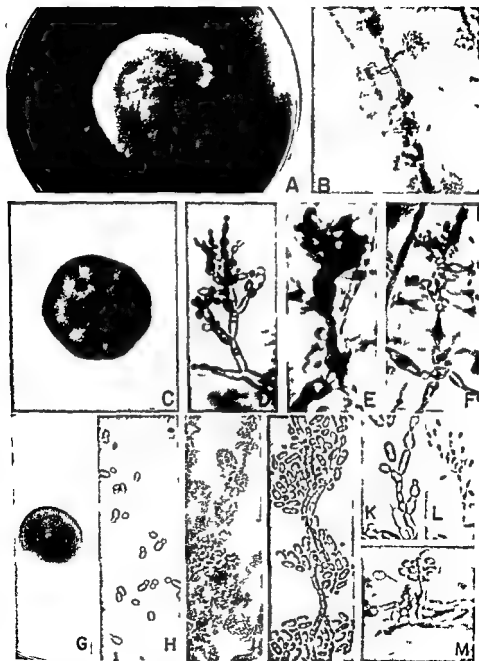
- A Colony on Sabouraud's glucose agar four weeks
- B Cup shaped conidiophores with spores $\times 400$

Fonsecaia pedrosoi

- C Colony on Sabouraud's glucose agar five weeks
- D F Three types of sporulation
 - D *Cladosporium* $\times 600$
 - L *Acrotheca* $\times 600$
 - F *Phialophora* $\times 600$

Fonsecaia dermatitidis

- G Colony on Sabouraud's glucose agar 23 days
- H Yeastlike phase note double budding cells $\times 800$
- I Tullularia phase showing clusters of spores along hyphae $\times 200$
- J Same $\times 500$
- K *Cladosporium* type of sporulation $\times 500$
- L Same $\times 600$
- M *Acrotheca* type of sporulation $\times 500$



FONSECAIA COMPACTA
CLADOSPORIUM CARRIONI

Figure 27

Fonsecaia compacta

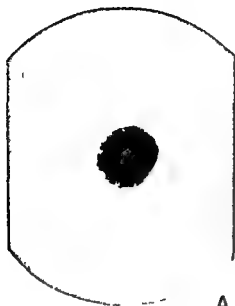
A Colony on Sabouraud's glucose agar six weeks

B Complex conical head X400

Cladosporium carrioni

C Colony on Sabouraud's glucose agar four weeks

D Cladosporium type of sporulation X400



A



B



C



D

CONTAMINANTS

SAPROPHYTIC FUNGI are a constant nuisance in laboratories engaged in medical mycologic work. They appear frequently on the usual plating media are often found in pathologic materials and are at times mistaken for pathogenic fungi. To learn to differentiate between the saprophytic and the pathogenic fungi is a fundamental requirement in the laboratory attempting mycologic examinations if serious mistakes are to be avoided in the diagnosis of fungus disease.

Recognition of contaminants should not be difficult provided one is familiar with a few of their common characteristics. The saprophytic fungi usually grow well at room temperature on the common mycologic media and poorly or not at all at 37° C. they are in general highly pigmented forming bluish green or green cream to yellow orange brown deep rose or black colonies and nearly always produce an abundance of characteristic reproductive structures. The pathogenic fungi on the other hand are commonly slow growing at room temperature or at 37° C. and often require special media for spore formation. There are some pathogenic fungi that form dark green or black pigments and some species of contaminants that are white or light colored. Therefore a fungus isolated from a pathologic specimen should not be discarded as a saprophyte because of its pigment without a thorough cultural examination and microscopic study; neither should an isolate because it is white be considered a pathogen without an equally thorough study.

Eighteen common contaminants encountered in the laboratory are described and illustrated in the following pages. The giant colonies of all these fungi were grown on Sabouraud's glucose medium at room temperature. Classification by genus is adequate in the diagnostic laboratory for the identification of contaminants. Photomicrographs to illustrate the microscopic structural characteristics by which these contaminants can be identified generically are presented. These structures are best demonstrated in slide culture preparations on Czapek's or corn meal agar.

PENICILLIUM SP
PAECILOMYCES SP

Figure 28

Penicillium sp

A Colony first growing, attaining a diameter of 52 mm in ten days flat with a powdery bluish green surface surrounded by a narrow white border

B Microscopically the diagnostic structure known as the "penicillus" (a) or brush is seen. This structure consists of chains of spores pinched off from flask shaped sterigmata (phialides) (b) borne in whorls from the ends of metulae (c) (short branches) arising from branched or unbranched conidiophores (d).

Many species of this genus differ widely in color, texture and rate of growth. Generic identification must be based upon demonstration of the "penicillus" or brush.

Paecilomyces sp

C Colony rapidly growing, attaining a diameter of 80 mm in five days flat with a surface of yellowish brown powdery mycelium

D Microscopically the conidial bearing structures (a) suggest *Penicillium* but differ in that the flask shaped sterigmata (b) are long and tubular bend away from the axis of the conidiophore (c) and are not always in whorls. Long, well separated chains of small elliptical conidia (d) are borne at the tips of the sterigmata.



SCOPULIARIOPSIS SP
CEPHALOSPORIUM SP

Figure 29

Scopulariopsis sp

A Colony first growing, attaining a diameter of 63 mm in eleven days flat at first white later light brown and powdery with a light tan periphery, undersurface brownish at center fading gradually to a light tan

B Microscopically the hyphae bear many short simple or branched conidiophores (a) which suggest the "penicillus" of *Penicillium*. The sterigmata (b) produce chains of lemon shaped conidia (c) with a somewhat pointed apex and a truncate base. The mature spores are echinulate.

Cephalosporium sp

C Colony moderately fast growing, attaining a diameter of 51 mm in seventeen days compact center slightly depressed irregularly folded cream colored felt like and surrounded by a slightly raised pure white border of finely powdery mycelium terminating in a thin long nipped mycelium reverse pale yellow

D Microscopically numerous elongate hyaline conidia borne in spherical clusters (a) at the tips of long or short lateral conidiophores (b)



A



B



C



D

TRICHOSPORON SP
RHODOTORULA SP

Figure 30

Trichosporon sp

A Colony moderately fast growing, attaining a diameter of 40 mm in thirteen days ivory membranous and radially folded center moist and veistlike

B Microscopically pseudomycelium and true mycelium are formed and many blastospores (a) and arthrospores (b)

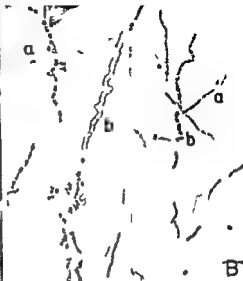
This fungus is often found as a contaminant in sputum
Formation of blastospores differentiates it from
Geotrichum

Rhodotorula sp

C Colony slow growing attaining a diameter of 15 mm in fourteen days deep coral in color low convex glistening soft and smooth occasionally becoming wrinkled

D Microscopically oval or round thin walled budding cells

The genus consists of at least seven species showing a wide variation in shape and size of cell and in the number of buds (one to several) produced by a single cell as well as in colony texture they produce carotenoid pigments (red orange or yellow) The cells form either no capsule or a faint one No true mycelium or ascospores are produced



MONILIA SITOPHILA STREPTOMYCES SP

Figure 31

Monilia sitophila

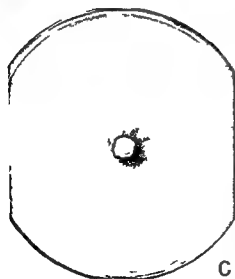
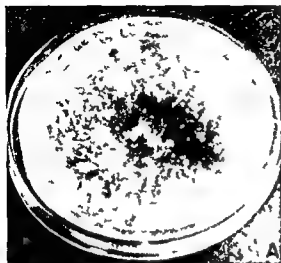
A Colony rapidly growing, often completely filling the Petri plate or culture tube within three or four days; white at first, growing close to the surface of the agar, quickly becoming floccose and showing salmon colored masses.

B Microscopically: branching chains of ovate conidia (a) borne on short conidiophores (b); thick walled arthrospores (c) formed by breaking up of the old hyphae into separate cells.

Streptomyces sp

C Colony slow growing, attaining a diameter of 5 mm in eleven days; rusted slightly folded hard leathery, closely adherent to medium and covered with a white aerial mycelium. Many species are variously pigmented: white, gray, red, rose, lavender, green, depending largely on the nature of the media and the conditions of growth. Characteristically they produce a strong, musty odor.

D Microscopically: long, branching, aerial hyphae (a) of 1 μ or less in diameter; from these hyphae arise spore bearing filaments (sporophores) which by segmentation form chains of spherical or oval spores (b). The sporophores may be straight, wavy, loosely or tightly spiraled.



FUSARIUM SP
CLADOSPORIUM (HORMODENDRUM) SP

FIGURE 32

Fusarium sp

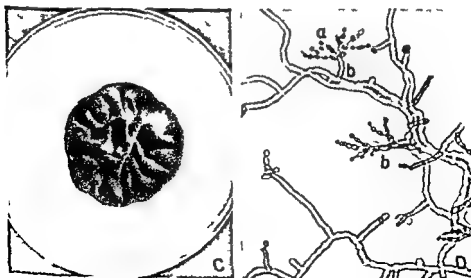
A Colony fast growing, attaining a diameter of 55 mm in seven days. At first white. Later violet with a center of heavy fluffy mycelium and a border of flat white mycelium. This genus consists of many species, some of which are of different colors: white, tan, pink, violet, or red.

B Microscopically: branched or unbranched conidiophores (a) on thin hyphae; the conidiophores produce two types of spores: macroconidia long multi-septate sickle-shaped (b); microconidia ovoid one celled (c).

Cladosporium (Hormodendrum) sp

C Colony moderately fast growing, attaining a diameter of 42 mm in seventeen days; irregularly heaped and folded with a smooth flat periphery; surface velvety olive green; undersurface greenish black. This fungus does not grow at 37° C. is do the pathogenic species of the same genus.

D Microscopically: mycelium olivaceous; conidia in branching tree-like chains (a); borne from branched conidiophores (b) of variable lengths.



PHOMA SP PULLULARIA SP

Figure 33

Phoma sp

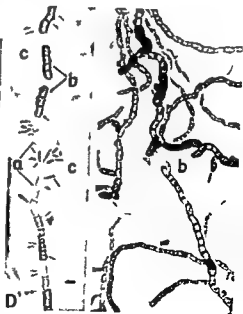
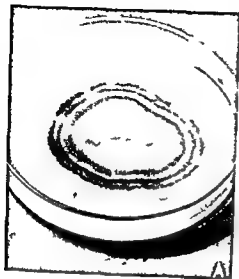
A Colony fast growing, attaining a diameter of 70 mm in twelve days consisting of two distinct zones: a central umbonated zone (35-40 mm) of gray to taupe aerial mycelium and a peripheral zone (14 mm wide) of shorter nipped brownish aerial hyphae with concentric darker shiny rings containing pycnidia border flat pinkish tan having in outermost layer of thin cream to tan submerged hyphae. Reverse shows a smudged brownish black pigment at center with beige border.

B Microscopically the identifying structures are the globose or flask shaped pycnidia (a) upon crushing these are found to be filled with many small ovate to clavate one celled hyaline pycnidiospores (b) that have been produced on short conidiophores inside the pycnidium. Some species of *Phoma* show pycnidia with one or more ostioles.

Pullularia sp

C Colony fast growing, attaining a diameter of 60 mm in fourteen days at first white flat and membranous later showing an irregular raised folded black shiny center of veastlike appearance surrounded by a flat greenish black border terminating in a fringe of greenish white mycelium.

D Microscopically hyphae of two types hyaline thin walled (a) when young, later dark and thick walled (b) conidia elliptical sometimes budding, (c) borne by repetition at various fertile points on mycelium.



HELMINTHOSPORIUM SP ALTERNARIA SP

Figure 34

Helminthosporium sp

A Colony moderately fast growing, attaining a diameter of 40 mm in fourteen days grayish green raised with surface of heavily matted wooly mycelium

B Microscopic ally hyphae light to dark giving rise to long septate simple or branched conidiophores (a) having a knotted twisted appearance; conidia brown ovoid containing four or more cells (b)

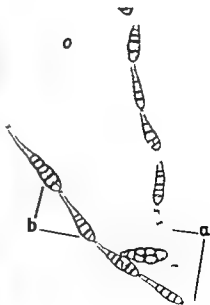
Alternaria sp

C Colony rapidly growing, attaining a diameter of 75 mm in nine days grayish white at first later greenish black surface wooly or felt like with loose grayish mycelial tufts border of grayish white mycelium

D Microscopic ally hyphae hyaline or dark conidiophores (a) short or elongate single or in groups brown or greenish brown conidia (b) brown transversely and longitudinally septate (mura form) borne singly or in acropetal chains



B



D

ASPERGILLUS SP SYNCEPHALASTRUM SP

Figure 35

Aspergillus sp

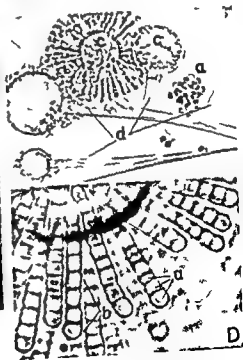
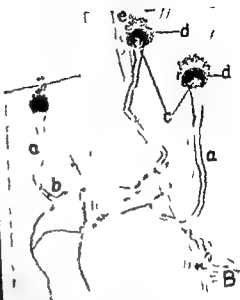
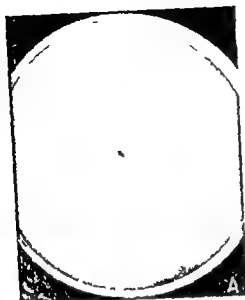
A Colony fast growing, attaining a diameter of 70 mm in seven days flat with a center of thin grayish blue green somewhat floccose matted mycelium and border of sparse partially submerged grayish mycelium reverse pale yellowish green

B Microscopically unbranched nonseptate conidiophore (a) arising from a specialized cell (b) in the mycelium (foot cell) at the apex of the conidiophore is the globose or elliptical vesicle (c) bearing phialides (d) (flask shaped structures) from which are produced chains of conidia (e)

Syncephalastrum sp

C Colony rapidly growing, quickly filling the Petri plate with a dense white fluffy aerial mycelium later dark gray

D Microscopically mycelium nonseptate Sporangiospores (a) borne serially within finger like sporangioles (b) arising radially on inflated terminal ends (c) of branchlike sporangiophores (d)



MUCOR SP RHIZOPUS SP

Figure 36

Mucor sp

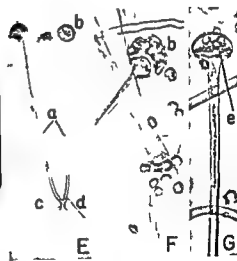
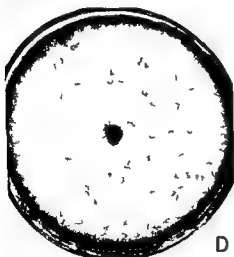
A Colony rapidly growing, filling Petri dish with floccose aerial mycelium first white later gray

B C Microscopically mycelium nonseptate rhizoids absent sporangiophores (1) branched or unbranched borne at any point on the hyphae terminating in large globose many spored sporangia (b) sporangial wall easily ruptured scattering ellipsoid or globose spores (c) and revealing columella (d) (a swollen end of sporangiophore)

Rhizopus sp

D Growth extremely rapid cottony grayish white aerial mycelium completely filling a Petri dish within three days

E C Microscopically mycelium nonseptate At the apex of a long erect unbranched sporangiophore (1) is borne the typical sporangium (b) a large spherical black spore case filled with many round brownish spores sporangiophores arise singly or in small groups directly opposite rhizoids (c) (root like structures) attached to the substrate and connected by hyphal branches (d) (stolons) running along the surface of the substrate The ruptured sporangium freed of spores reveals columella (e)



Media

Beef Infusion Broth with Glucose

Beef	450.0 gm
Leptone (Difco's proteose)	20.0 gm
Sodium chloride	5.0 gm
Glucose	10.0 gm
Distilled water	1000.0 ml

Beef Infusion Agar with Glucose

Beef	450.0 gm
Distilled water	500.0 ml

Agar	17.5 gm
Pepton	10.0 gm
Sodium chloride	5.0 gm
Distilled water	500.0 ml
Glucose	10.0 gm

Beef Heart Infusion Agar with Blood

Beef heart	450.0 gm
Distilled water	500.0 ml

Agar	20.0 gm
Sodium chloride	5.0 gm
Pepton	10.0 gm
Distilled water	500.0 ml

Horse, sheep or rabbit blood defibrinated sterile 50 ml per 900 ml

Beef Infusion Agar with Blood and Antibiotics

Beef heart infusion agar or dehydrated blood agar base	150.0 ml
--------------------------------------------------------	----------

Horse blood defibrinated sterile	10.0 ml
Penicillin (50 units per ml of medium)	8000.0 units
Streptomycin (50 units per ml of medium)	8000.0 units

Glucose Cysteine Yeast Infusion Agar

Yeast infusion	1000.0 ml
Peptone	10.0 gm
NaCl	5.0 gm
Agar	20.0 gm
Cysteine or cysteine hydrochloride	1.0 gm

Glucose Cystine Yeast Infusion Agar (cont.)

Glucose 20% sterile solution	500 ml
Blood (rabbit or horse)	500 ml
Combine and heat at 60° for three hours	

Potato Infusion Agar with Glucose †

Potatoes peeled and ground	2000 gm
Distilled water	10000 ml
Agar	200 gm
Glucose	200 gm
Used unadjusted at pH 5.6-5.8 or adjusted and sequent to sterilization to pH 3.5 with a sterile 10% tartaric acid	

*Corn Meal Agar **

Agar	17.50 gm
Corn meal yellow ground	40.00 gm
Distilled water	1000.00 ml
Dissolve the agar in one half the water by autoclaving. Add the corn meal to the remainder of the water and heat it approximately 65° for one hour. Filter through paper. Add the dissolved agar and the corn meal filtrate (300-400 ml) and filter through cotton. Dispense as required and autoclave twenty minutes. No pH adjustment is required.	

Campy's Agar

NaNO ₂	2.00 gm
K ₂ HPO ₄	1.00 gm
NaNO ₃ 7H ₂ O	0.50 gm
KCl	0.50 gm
FeSO ₄	0.01 gm
Sucrose	0.00 gm
Agar	15.00 gm
Distilled water	1000.00 ml

pH 6.6

Rice Medium

Rice white grain	0 kg
Distilled water	250 l
Place rice and water in 100 ml flask with magnetic bar, incubated at 140° r bottom of flask at evening in the fermenter and autoclave twenty minutes.	

Salt Agar's Glucose Broth

Bacto-Leptone	10.0 g
Glucose	10.0 g
Distilled water to make final pH 5.2	1000.0 ml

Sabouraud's Glucose Agar 1

Agar	20.0 gm
Peptone	10.0 gm
Glucose technical	10.0 gm
Distilled water to make	1000 ml
Adjusted subsequent to sterilization to pH 5.6-5.8 with sterile 10% tartaric acid	

Sabouraud's Peptone Agar with Honey

Agar	1
Peptone	1
Honey	1
Distilled water to make	100
Adjusted subsequent to sterilization to pH 5.6-5.8 with sterile 10% tartaric acid	

Carbohydrate Broth for Fermentation Tests with Candids

Difco beef extract	
Sodium chloride	
Difco peptone	1
Distilled water	999
Heat to boiling and titrate exactly to pH 7.2. Add 100 ml of indicator solution, filter and dispense in 10-ml quantities in Durham type fermentation tube. Autoclave at 15 pounds pressure for exactly 15 minutes. Add 0.5 ml of a 20 per-cent solution of the carbohydrate. The broth should not be kept for more than two or three weeks because slight changes in pH may occur.	

Indicator solution

Brom thymol blue	0.01 gm
Distilled water	100.00 ml

Add a small amount of 1N NaOH to make the solution alkaline. When indicator is in solution neutralize with 1N HCl until the exact neutral point is reached and one drop of either acid or alkali will cause a complete change of color.

(Martin D. S. et al. J. Bact. 34:99, 1937)

Wort Agar

Agar	20.00 gm
Ammonium chloride NH_4Cl	1.00 gm
Leptone	0.78 gm
Maltose (technical)	12.75 gm
Malt extract	15.00 gm
Dextrin	2.75 gm
Glycerol	2.33 gm
Dipotassium phosphate K_2HPO_4	1.00 gm
Distilled water	1000.00 ml

Formula are based on the weight of the ingredients of the medium of Laboratory A. I. T. E. Walworth A. B. Standard Method of the Division of Laboratory A. I. T. E. New York State Department of Health, Division of Bacteriology, New York City, 1947.

† The medium is prepared in the laboratory of the Division of Bacteriology.

References

GENERAL

- Mycologie Médicale Communications et rapports présentés aux Journées de Mycologie Médicale (14-15 décembre 1956) organisées par l'Institut Pasteur et la Société Française de Mycologie Médicale* Paris L'Expansion Scientifique Française 314 p
- Ainsworth G C *Medical Mycology An Introduction to its Problems* New York Pitman 1952 105 p
- Ajello L Collecting specimens for the laboratory demonstration and isolation of fungi *J Amer Med Assoc* 146 1581 1951
- Ajello L Cant A Q and Gubke M A The effect of tubercle concentration on the growth of fungi causing pulmonary mycoses *J Lab & Clin Med* 39 496 1951
- Alexopoulos C J *Introductory Mycology* New York Wiley 1952 p 312-338
- Ash J E and Spitz S *Pathology of Tropical Diseases An Atlas* Philadelphia Saunders 1915 350 p
- Buell C B and Weston W H Application of the mineral oil conservation method to mounting collections of fungous cultures *Amer J Botany* 34 525 1947
- Cochrane V W *Physiology of Fungi* New York Wiley 1953 524 p
- Conant V F et al *Manual of Clinical Mycology* 2d ed Philadelphia Saunders 1954 456 p
- Cook A H editor *The Chemistry and Biology of Yeasts* New York Academic Press 1958 763 p
- Dodge C W *Medical Mycology Fungous Diseases of Men and Other Mammals* St Louis Mosby 1935 900 p
- Emmons C W *Mycoses of animals* In Brandly C A and Jungherr E L editors *Advances in Veterinary Science* Vol II New York Academic Press 1953 p 47-63
- Gordon M A A key to the human mycoses *J Bact* 63 385 1952
- Girdley M F A stain for fungi in tissue sections *Amer J Clin Path* 33 303 1953
- Grocott R G A stain for fungi in tissue section and smears Using Gomori's methenamine-silver nitrate technique *Amer J Clin Path* 25 975 1955
- Hawker L E *The Physiology of Reproduction in Fungi* Cambridge (England) Univ Press 1957 128 p
- Jillson M F *Mycology* New England J Med 249 523 581 1953
- Kennedy E L *Practical Medical Mycology* Springfield Illinois Thomas 1955 145 p
- Kligman A M and DeLamater E D The immunology of the human mycoses *Ann Rev Microbiol* 4 283 1950
- Kligman A M Meson H and DeLamater E D The histopathologic diagnosis of fungus diseases *Amer J Clin Path* 21 86 1951

- Kurink J M The examination of sputum I Collection and detection II Search for elastic tissue III Search for fungal spores *Amer Rev Tuberc* 76:671-675 679 1957
- Lacaz C de Silva *Manual de Micologia Medica* 2d ed Sao Paulo Brazil Impos Dupont 1956 422 p
- Langeron M and Vanbrenghem R *Précis de Mycologie* 2d ed Paris Masson 1952 703 p
- Lewis C M et al *An Introduction to Medical Mycology* 4th ed Chicago Yr Bk Pub 1959 453 p
- Lodder J and Kreger Van Rij N J W *The Yeasts A Taxonomic Study* New York Interscience 1952 713 p
- Moss E S and McQuinn A L *Atlas of Medical Mycology* Baltimore Williams & Wilkins 1953 215 p
- Nickerson W J Medical mycology *Ann Rev Microbiol* 7:215 1953
- Pelezar M J Jr and Reid R D *Microbiology* New York McGraw Hill 1955 p 141 156
- Riddell B W Fungal diseases of Britain *Brit Med J* 2:53 1956
- Ridzell B W Permanent stained mycological preparations obtained by alkali culture *Mycologia* 1:265 1950
- Skinner C F Emmon C W and Tsuchiya H M *Henriks Molds Yeasts and Actinomyces* 2d ed New York Wiley 1947 109 p
- Smith D T *Fungal Diseases of the Lungs* Springfield Illinois Thomas 1917 59 p
- Smith D T Mucellineous fungus diseases *J Chronic Dis* 5:55 1957
- Widworth A B *Standard Methods of the Division of Laboratories and Research* New York State Department of Health 3rd ed Baltimore Williams & Wilkins 1947 p 166-178
- Weed L A Techniques for the isolation of fungi from tissues obtained at operation and necropsy *Amer J Clin Path* 29:496 1958
- Wilson J W *Clinical and Immunologic Aspects of Fungal Diseases* Springfield Illinois Thomas 1957 250 p
- Zimmerman I F Fungal fungus infections complicating other diseases *Ann J Clin Path* 25:46 1955

- Benham R W Nutritional studies of the dermatophyte—Effect on growth and morphology with special reference to the production of microconidia *Tr New York Acad Sci* 15 102 1953
- Bucolo F C and Benham R W Pigment production in the differentiation of *Trichophyton mentaglyphes* and *Trichophyton rubrum* *Mycologia* 41 91 1949
- Conant V F Statistical analysis of spore size in genus *Microsporum* *J Invest Dermat* 4263 1941
- Drouhet E Recherches sur la nutrition de dermatophytes II Action des acides aminés sur la croissance et la morphogenèse *Ann Inst Pasteur* 82 348 1957
- Drouhet E Recherches sur la nutrition des dermatophytes III L'histidine facteur de croissance des *Trichophyton* du groupe *rosaceum* *Ann Inst Pasteur* 85 791 1953
- Drouhet E and Marvat F Recherches sur la nutrition des dermatophytes I Etude de besoins vitaminiques *Ann Inst Pasteur* 82 338 1956
- Emmons C W Dermatophytes: natural grouping based on the form of the spores and accessory organs *Arch Dermat & Syph* 30 337 1931
- Fowler L P and Georg L K Suppurative ringworm contracted from cattle *Arch Dermat & Syph* 56 780 1947
- Georg L K The relation of nutrition to the growth and morphology of *Trichophyton fauiforme* *Mycologia* 42 683 1950
- Georg L K The relationship between the downy and granular forms of *Trichophyton mentaglyphes* *J Invest Dermat* 23 123 1954
- Georg L K *Trichophyton tonsurans* ringworm—A new public health problem *Pub Health Rep* 67 53 1952
- Georg L K Use of a cycloheximide medium for isolation of dermatophytes from clinical materials *AMA Arch Dermat & Syph* 67 355 1953
- Georg L K and Camp L B Routine nutritional tests for the identification of dermatophytes *J Bact* 74 113 1957
- Gordon M A The occurrence of the dermatophyte *Microsporum gypsum* as a saprophyte in soil *J Invest Dermat* 20 201 1953
- Hazen E L Effect of temperature and nutrition upon macroconidial formation of *Microsporum audouinii* *Mycologia* 49 11 1957
- Hazen E L *Microsporum audouinii*: The effect of yeast extract, thiamine, pyridoxine and *Bacillus uedemannensis* on the colony characteristics and macroconidial formation *Mycologia* 39 200 1947
- Kligman A M Tinea capitis due to *M audouinii* and *M canis* II Dynamics of the host-parasite relationship *AMA Arch Dermat* 71 313 1955
- Langeron M and Mikolouchet S Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides Essai de classification *Ann. Parasitol* 8 465 1930
- Saloua J M *Maladies du Cuir Chevelu* III Les Maladies Cryptogamiques Les Teignes Paris Masson 1910 855 p
- Silva M Nutritional studies of the dermatophytes—Factors affecting pigment production *Tr New York Acad Sci* 15 106 1953
- Silva M and Benham R W Nutritional studies of the dermatophytes with special reference to the red pigment producing varieties of *Trichophyton mentaglyphes* *J Invest Dermat* 22 285 1954
- Silva M Kesten B M and Benham R W *Trichophyton rubrum* infections: A clinical, mycologic and experimental study *J Invest Dermat* 25 311 1955

ACTINOMYCOSIS

- Emmons C W. *Actinomyces* and actinomycosis. *Puerto Rico J Pub Hlth & Trop Med* 11 63 1935
- Emmons C W. The isolation of *Actinomyces bovis* from tonsillar granules. *Pub Health Rep* 53 1967 1938
- Frisson D. *Pathogenic Anaerobic Organisms of the Actinomyces Group*. London: His Majesty's Stat Off. 1940. 63 pp.
- Erickson B and Porteous J W. Commentaries in pathogenic anaerobic *Actinomyces* cultures. *J Gen Microbiol.* 13 281 1955
- Hazen E L and Little C N. *Actinomyces bovis* and "anaerobic diphtheroid". Pathogenicity for humans and other differentiating characteristics. *J Lab & Clin Med* 51 968 1958
- King S and Meyer F. Metabolic and serologic differentiation of *Actinomyces bovis* and "anaerobic diphtheroids". *J Bact* 74 234 1957
- Meyer E and Vergey P. Mouse pathogenicity as a diagnostic aid in the identification of *Actinomyces bovis*. *J Lab & Clin Med* 36 667 1950
- Negróni I and Bonfiglioli H. Morphology and biology of *Actinomyces israeli* (Kirse). *J Trop Med & Hyg* 40 226 240 1937
- Leahy J W and Scribner J H. Actinomycosis and nocardiosis. *J Chron Dis* 5 374 1957
- Rosebury T, Epps L J and Clark A. A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. *J Infect Dis* 74 131 1944
- Suter L S. Evaluation of criteria used in the identification of *Actinomyces bovis* with particular reference to the catalase reaction. *Mycopath et Mycol Appl* 20 1956
- Thompson L. Isolation and comparison of *Actinomyces* from human and bovine infections. *Proc Staff Meet Mayo Clinic* 25 81 1950
- Weed L A and Biggenstoss A H. Actinomycosis: A pathologic and bacteriologic study of twenty-one fatal cases. *Amer J Clin Path* 19 201 1949
- Wilson G S and Miles A A. *Topley and Wilson's Principles of Bacteriology and Immunity*. 4th ed. Vol 1. Baltimore: Williams & Wilkins. 1953. p. 157-478
- Wright J H. The blocks of the microorganisms of actinomycosis. *J Med Research* 13 349 1905

NOCARDIOSIS

- Conant A F. Medical Mycology. *Nocardia* (Aerobic Actinomycetes). In D. F. J. *Bacterial and Mycotic Infections of Man and Animals*. Lippincott 1955. p. 581-589
- Gonzalez Ochua A. Estudio comparativo entre *Actinomyces mexicanus* y *Israeliensis* y *A. asteroides*. *Rev Inst Salub y Enfren Trj* 6 15 1955
- Gonzalez Ochua A and Sandoval M A. Características de los actinomicetos patógenos más comunes. *Rev Inst Salub y Enfren Trj* 16 143 1955
- Cordell R F and Smith M M. Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. *J Bact* 69 147 1955

- Mackinnon J E and Artigas ytia Allende H C The main species of pathogenic aërolic actinomycetes causing mycetomas *Tr Roy Soc Trop Med & Hyg* 50 31 1956
- Mariat F Physiologie des actinomycetes aerobies pathogenes *Mycopath et Mycol Appl* 9 111 1953
- Waksman S A *The Actinomycetes Their Nature Occurrence Activities and Importance* Waltham Mass Chronica Botanica Co 1950 230 p
- Wilson G S and Miles A A *Topley and Wilson's Principles of Bacteriology and Immunity* 4th ed Vol 1 Baltimore Williams & Wilkins 1955 ■ 457-478

BLASTOMYCOSIS

- Curtis A C and Bocobo F C North American blastomycosis *J Chronic Dis* 5 404 1957
- Halliday W J and McCoy E Biotin as a growth requirement for *Blastomyces dermatitidis* *J Bact* 70 464 1955
- Layton J M McKee A P and Stamler F W Dual infection with *Blastomyces dermatitidis* and *Histoplasma capsulatum* *Amer J Clin Path* 23 904 1953
- Manwaring J H Unusual forms of *Blastomyces dermatitidis* in human tissues *Arch Path* 48 421 1949
- Nickerson W J Physiological bases of morphogenesis in animal disease fungi *Tr New York Acad Sci* 13 140 1951
- Salvin S B Phase-determining factors in *Blastomyces dermatitidis* *Mycologia* 41 311 1949
- Selwarz J and Baum G L Blastomycosis *Amer J Clin Path* 21 999 1951
- Smith J G Jr Harris J S Conant V F and Smith D T An epidemic of North American blastomycosis *J Amer Med Assoc* 158 641 1953
- Tompkins V and Schleifstein J Small forms of *Blastomyces dermatitidis* in human tissues *AMA Arch Path* 55 432 1953
- Weed, L A North American blastomycosis *Amer J Clin Path* 25 37 1955

SOUTH AMERICAN BLASTOMYCOSIS

- Almeida F de Formas pequenas de *P. brasiliensis* *B. dermatitidis* e *H. capsulatum* nos tecidos *An Fac Med Univ S Paulo* 28 141 1954
- Ponseca J ■ Blastomycose Sul Americana Estudo das lesões dentarias e para dentarias sob o ponto de vista clínico e histopatológico Tese apresentada à Faculdade de Farmácia e Odontologia da Universidade de São Paulo S O Paulo Gráfico Político Ltda 1957 182 p
- Furtado T A Wilson J W and Plunkett O A South American blastomycosis or paracoccidiodomycosis The mycosis of Lutz Splendore and Almeida *AMA Arch Dermat & Syph* 70 166 1954
- Lacaz C da Silva South American blastomycosis *An Fac Med Univ S Paulo* 29 1 1955 56
- Ferry H O Weed L A and Keland H R South American blastomycosis Report of case and review of laboratory features *AMA Arch Dermat & Syph* 70 477 1954

MONILIASIS

- Ajello L A A simple method for preparing corn meal agar *Mycologia* 57: 630 1945
- Benham R W Species of *Candida* most frequently isolated from man: Methods and criteria for their identification *J Chron Dis* 5:460 1957
- Drouhet F and Vuot M Facteurs vitaminiques de croissance des *Candida* *Ann Inst Pasteur* 92:825 1957
- Gordon M A Differentiation of yeasts by means of fluorescent antibody *Proc Soc Exper Biol & Med* 97:694 1958
- Gordon M A Bradley L G and Grant V Q The influence of different types of corn meal agar upon chlamydospore production of *Candida albicans* *J Lab & Clin Med* 40:316 1952
- Higman A M Aids in technique in the identification of *Candida albicans* *J Invest Dermat* 14:173 1950
- Nickerson W J and Muskowsky Z A polysaccharide medium of known composition favoring chlamydospore formation in *Candida albicans* *J Infect Dis* 92:20 1953
- Pollock J D and Benham R W The chlamydospores of *Candida albicans*: Comparison of three media for their induction *J Lab & Clin Med* 50:313 1957
- Rid J D Jones M M and Carter E B A simple clear medium for demonstration of chlamydospores of *Candida albicans* *Amer J Clin Path* 23:935 1953
- Skinner C F The yeast like fungi: *Candida* and *Pectonomyces* *Bact Rev* 11:227 1947
- Taschdjian C L Routine identification of *Candida albicans*: Current methods and a new medium *Mycologia* 49:332 1957
- Wickerham L J Apparent increase in frequency of infection involving *Torulopsis glabrata*: Procedure for its identification *J Amer Med Assoc* 165:47 1957

COCCIDIOIDOMYCOSIS

- Crestz J R and Luckett T F A method for cultural identification of *Coccidioides immitis* *Amer J Clin Path* 24:1318 1954
- Emmons C W Biology of *Coccidioides* In Nickerson W J *Pathogenic Fungi* Waltham Mass: Chronica Botanica Co 1957 p 715
- Fiese M J *Coccidioidomycosis* Springfield Illinois: Thomas 1958 253 p
- Friedman L and Pappagianis D The inhibitory effect of peptone on the sporulation of three strains of *Coccidioides immitis* *Amer Rev Tuberc* 4: 147 1956
- Friedman L Pappagianis D Berman R J and Smith C E Studies on *Coccidioides immitis*: Morphology and sporulation capacity of fifty-seven strains *J Lab & Clin Med* 47:435 1953
- Friedman L Smith C E Roessler W C and Berman R J The virulence and infectivity of twenty-seven strains of *Coccidioides immitis* *Amer J Hyg* 67:198 1956

- Georg L K, Ajello L and Gordon M A A selective medium for the isolation of *Coccidioides immitis* *Science* 114:387 1951
- Huppert M and Walker L J The selective and differential effects of cycloheximide on many strains of *Coccidioides immitis* *Amer J Clin Path* 29:291 1959
- Smith D T and Hurrell E R Jr Fatal coccidioidomycosis A case of a laboratory infection *Amer Rev Tuberc* 57:368 1918
- United States Public Health Service Communicable Disease Center Proceedings of Symposium on Coccidioidomycosis Phoenix Arizona February 11-13 1957 Washington D C Public Health Service Publication No 575 1957 197 p

CRYPTOCOCCOSIS

- Benham R W Cryptococcosis and blastomycosis *Ann New York Acad Sci* 50:1-99 1950
- Benham R W The genus *Cryptococcus* *Bact Rev* 20:189 1956
- Cox L B and Tollhurst J C Human Torulosis A Clinical Pathological and Microbiological Study with a Report of Thirteen Cases Melbourne Australia Melbourne Univ Press 1946 149 p
- Drouhet E, Ségrétain G and Aubert J P Polyonide capsulaire d'un champignon pathogène *Torulopsis neoformans* Relation avec la virulence *Ann Inst Pasteur* 70:891 1950
- Emmons C W *Cryptococcus neoformans* strains from a severe outbreak of bovine mastitis *Mycopath et Mycol Appl* 6:31 1955
- Emmons C W Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*) *Amer J Hyg* 62:2-7 1955
- Evans E E and Hurrell E R Jr Cryptococcosis (Torulosis) A review of recent cases *Univ Michigan Med Bull* 18:43 1952
- Kao C J and Schwarz J The isolation of *Cryptococcus neoformans* from pigeon nests With remarks on the identification of virulent cryptococci *Amer J Clin Path* 27:652 1957
- Littman M L Capsule synthesis by *Cryptococcus neoformans* *Tr New York Acad Sci* 20:6-9 1958
- Littman M L An improved method for detection of urea hydrolysis by fungi *J Infect Dis* 101:51 1957
- Littman M L and Zimmerman, L F *Cryptococcosis Torulosis or European Blastomycosis* New York Grune & Stratton 1956 205 p
- Seeliger H P R Use of a urease test for the screening and identification of cryptococci *J Bact* 72:127 1956
- Wil on J W Cryptococcosis (Torulosis European blastomycosis Basse-Buschke's disease) *J Chronic Dis* 5:445 1957

GEOTRICHOSIS

- Bendove M A and Ashe B I *Geotrichum septicemia* Report of a case *AMA Arch Int Med* 89:107 1952

- Carmichael J W. *Geotrichum candidum*. *Mycologia* 49 6-10 1957
- Kaliski S R, Beene M L and Mattman L. *Geotrichum* in blood stream of an infant. *J Amer Med Assoc* 159 1-07 1953
- Kunstadter H H, Milzer A and Whitcomb F. Bronchopulmonary geotrichosis in children. *Amer J Dis Child* 79 82 1950
- Minton H, Young R V and Shanbrom E. Endobronchial geotrichosis. *Ann Int Med* 40 340 1954
- Smith D T. *Fungus Diseases of the Lung*. Springfield Illinois: Thomas 1947 p 16-19
- Thyotta Th and Urdil K. A family endemic of geotrichosis pulmonum. *Acta Path et Microbiol Scand* 26 673 1949
- Webster H H. Pulmonary geotrichosis. *Amer Rev Tuberc* 76 286 1957

HISTOPLASMIOSIS

- Proceedings of the Histoplasmosis Seminar Cincinnati Ohio Feb 11 1958* sponsored by the Jewish Hospital Association Cincinnati. Published by the Association (mimeo) 1958 100 p
- Ajello L. Geographic distribution of *Histoplasma capsulatum*. *Mykosen* 114 1958
- Binford C H. Histoplasmosis. Tissue reactions and morphologic variations of the fungus. *Amer J Clin Path* 25 25 1955
- Campbell C C. Reverting *Histoplasma capsulatum* to the yeast phase. *J Bact* 54 263 1947
- Conant V F. A cultural study of the life cycle of *Histoplasma capsulatum*. Darling 1906. *J Bact* 41 563 1941
- Drouhet F. Quelques aspects biologiques et mycologiques de *Histoplasma*. *Semaine des Hopitaux Paris* 33 1299 1957
- Drouhet F and Schwarz J. Croissance et morphogénèse d'*Histoplasma*. I. Etude comparative des phases mycelienne et levure d'18 souches d'*Histoplasma capsulatum* d'origine Américaine et Africaine. *Ann Inst Pasteur* 90 114 1956
- Duncan J T. Tropical African histoplasmosis. *Tr R egal Soc Trop Med & Hyg* 5-468 1958
- Edwards C A, Edwards M H and Hazen F L. Electron microscopic study of *Histoplasma* in mouse spleen. *J Bact* 17 129 1959
- Edwards M R, Hazen F L and Edwards C A. The fine structure of the yeast like cells of *Histoplasma* in culture. *J Gen Microbiol* 20 140 1959
- Fennons C W. The significance of saprophytism in the epidemiology of the mycoses. *Tr New York Acad Sci* 17 157 1954
- Furcolovs M L. Recent studies on the epidemiology of histoplasmosis. *New York Acad Sci* 2-127 1958
- Howell A. The efficiency of methods for the isolation of *Histoplasma capsulatum*. *Pub Health Rep* 63 173 1948
- Kurung J M. The isolation of *Histoplasma capsulatum* from patients. *Am Rev Tuberc* 66 578 1952
- Littman M L. Liver pleural glomeruloma associated with *Histoplasma capsulatum* and other fungi. *Amer J Clin Path* 25 1149 1955

- Lopez Fernandez J R Acción patogénica experimental de la levadura *Tarulopsis glabrata* (Anderson 1917) ladder y de vries 1938 productora de lesiones histopatológicas semejantes a las de la histoplasmosis. *An Fac Med Montevideo* 37:470 1932
- Pine L. and Peacock C L Studies on the growth of *Histoplasma capsulatum* IV Factors influencing conversion of the mycelial phase to the yeast phase. *J Bact* 75:167 1953
- Salvin H B Cysteine and related compounds in the growth of the yeastlike phase of *Histoplasma capsulatum*. *J Infect Dis* 84:75 1949
- Scherr G H Studies on the dimorphism of *Histoplasma capsulatum* 1 The roles of SH group and incubation temperature. *Exper Cell Res* 12:9 1957
- United States Public Health Service: *Proceedings of the Conference on Histoplasmosis* Excelsior Springs, Missouri, Nov. 18-20, 1952. Washington, D.C. Gov. Printing Office (Pub. Health Monograph No. 39) 1953. 32 pp.
- Vanreuselghem R *Histoplasma duboisii* and large forms of *Histoplasma capsulatum*. *Mycologia* 49:264 1956
- Weed L A and Parkhill E M The diagnosis of histoplasmosis in ulcerative disease of the mouth and pharynx. *Amer J Clin Path* 18:130 1918

ASPERGILLOSIS

- Enjalbert L, Segrain G, Eschajasse H, Morcau G and Bourdin M Deux cas d'aspergillose pulmonaire. Etude anatomo-pathologique. *Semaine des Hôpitaux Paris* 33:1 1957
- Hinson, K. F. W., Moon A. J. and Plimmer N. S. Broncho-pulmonary aspergillosis: a review and a report of eight new cases. *Thorax* 7:317 1952
- Kirschstein R L. and Sidransky H. Mycotic endocarditis of the tricuspid valve due to *Aspergillus flavus*. *AMA Arch Path* 62:103 1956
- Segrains G. and Vieu M. Formes parasitaires des *Aspergillus* dans l'aspergillose bronchique. Diagnostic biologique des aspergilloses broncho-pulmonaires. *Semaine des Hôpitaux Paris* 33:1-31 1957
- Stuart H. A. and Blink F. Aspergillosis of the ear. A report of twenty nine cases. *Can Med Assoc J* 72:334 1955
- Thom C. and Raper K. B. *A Manual of the Aspergill*. Baltimore: Williams and Wilkins 1945. 373 p.
- Vellios F., Crawford A. S., Gatzimos C. M. and Hynd H. Bronchial aspergillosis occurring as an intracavitary fungus ball. *Amer J Clin Path* 27:68 1957
- Ziskind J., Pizzolito P. and Buff E. E. Aspergillosis of the brain. Report of a case. *Amer J Clin Path* 29:554 1958

MUCORMYCOSIS

- Baker H. D. The diagnosis of fungus diseases by biopsy. *J Chronic Dis* 5:52 1957
- Baker R. D. Mucormycosis. A new disease? *J Amer Med Assoc* 163:805 1957

- Baker R D Pulmonary mucormycosis *Amer J Path* 3: 57 1958
 Bauer H Ajello L Adams E and Hernandez D U Cerebral mucormycosis
 Pathogenesis of the disease *Amer J Med* 18: 52, 1955
 Gregory J E Coklen A and Haymaker W Mucormycosis of the central
 nervous system A report of three cases *Bull Johns Hopkins Hosp* 73: 405
 1943
 Kuttan I Cerebral mucormycosis *J Clin Path* 14: 1954

MADUROMYCOSIS

- Ajello L The isolation of *Allischeria boydii* Shur an etiologic agent of mycetomas from soil *Amer J Trop Med & Hyg* 12: 170 1952
 Benjamin R W and Coyle L A *Allischeria boydii* causative agent in a case of meningitis *J Invest Dermat* 10: 99 1949
 Burns F L Moss F S and Bruce J W Mycetomycosis in the United States and Canada With a report of three cases originating in Louisiana *Amer J Clin Path* 15: 35 1945
 Emmons C W *Allischeria boydii* and *Monosporium aspersum* *Mycologia* 36: 169 1944
 Mackinnon J F A contribution to the study of the causal organisms of maduromycosis *Tr Roy Soc Trop Med & Hyg* 45: 470 1951
 Mackinnon J E Ferrada Ortega L V and Montemayor I *Madurella grisea* n. sp. A new species of fungus producing the black variety of maduromycosis in South America *Mycopathologia* 4: 394 1949
 Nishimura I Black grain maduromycosis caused by *Madurella grisea* Report of the first North American case and its response to therapy with diuretic phenylulfone *AMA Arch Dermat* 72: 530 1955
 Sarrasin G Diagnostic histologique des maduromycoses *Semaine d'Hôpital* Paris 33: 951 1957

SPOROTRICHOSIS

- Arthur C W and Albrittain J W Disseminated cutaneous sporotrichosis with systemic involvement *AMA Arch Dermat* 77: 157 1954
 Benjamin R W and Keeton B Sporotrichosis its transmission to the animal *J Infect Dis* 50: 437 1932
 Campbell C C Use of Francis glucose cystine blood agar in the laboratory cultivation of *Sporotrichum schenckii* *J Bact* 50: 233 1955
 Catinella I M Spohrer L W and Hynes F Sporotrichosis in the human cases among birds *J Amer Med Assoc* 117: 1074 1941
 Hopkins J G and Benjamin R W Sporotrichosis in New York State *New York State J Med* 32: 593 1932
 Mikkelson W M Brandt R L and Harrell F R Sporotrichosis A report of 12 cases including two with skeletal involvement *Ann Int Med* 47: 43 1957
 Slemaker F H et al Lymph meningitis due to *Sporotrichum schenckii* *AMA Arch Path* 61: 22 1957

Union of South Africa Transvaal Mine Medical Officers Association. *Sporotrichosis Infection on Mines of the Witwatersrand. A Symposium*. Johannesburg, S Africa Transvaal Chamber of Mines 1947. 67 p

CHROMOBLASTOMYCOSIS

- Binford C H, Hiss G and Emmons C W. Chromoblastomycosis. Report of a case from continental United States and discussion of the classification of the causative fungus. *Arch Dermat & Syph* 49:399 1944
- Binford C H, Thompson R K and Gorham M E. Mycotic brain abscess due to *Cladosporium trichoides* a new species. *Amer J Clin Path* 22:535 1952
- Brumpt E. *Précis de Parasitologie* 3rd ed Paris Masson 1922. 679 p
- Carrion A L. Chromoblastomycosis. *Ann New York Acad Sci* 50:1255 1950
- Carrion A L. Yeast like dematiaceous fungi infecting the human skin. Special reference to so called *Hormium dermatitis*. *Arch Dermat & Syph* 61:996 1950
- Carrion A L and Silva M. Chromoblastomycosis and its Etiologic Fungi. In Nickerson W J. *Biology of Pathogenic Fungi*. Waltham Mass. Chronica Botanica Co. 1947. 20-64 p
- Conant N F. The occurrence of a human pathogenic fungus as a saprophyte in nature. *Mycologia* 29:597 1937
- King A B and Collitt T S. Brain abscess due to *Cladosporium trichoides*. Report of the second case due to this organism. *Bull Johns Hopkins Hosp* 91:298 1952
- Martin D S, Baker R D and Conant N F. A case of verrucous dermatitis caused by *Hormodendrum pedrosi* (chromoblastomycosis) in North Carolina. *Amer J Trop Med* 16:593 1936
- McGill H C Jr and Brueck J W. Brain abscess due to *Hormodendrum* species. Report of third case. *AMA Arch Path* 62:303 1956
- Medlar E M. A new fungus *Phialophora terrucosa* pathogenic for man. *Mycologia* 200 1915
- Segretain G, Mariat F and Drouhet E. Sur *Cladosporium trichoides* isolé d'une mycose cérébrale. *Ann Inst Pasteur* 89:465 1955
- Silva M. The parietic phase of the fungi of chromoblastomycosis. Development of sclerotic cells in vitro and in vivo. *Mycologia* 49:318 1957
- Silva M. The saprophytic phase of the fungi of chromoblastomycosis. Effect of nutrients and temperature upon growth and morphology. *Tr New York Acad Sci* 21:46 1959
- Simson F W, Harrington C and Barnett J. Chromoblastomycosis. A report of six cases. *J Pathol & Bact* 55:191 1943
- Trejos A. *Cladosporium carrionii* n. sp. and the problem of *Cladosporia* isolated from chromoblastomycosis. *Rev Biol Trop* 2(1):75 1954

CONTAMINANTS

- Barnett H L. *Illustrated Genera of Imperfect Fungi*. Minneapolis. Burgess 1955. 118 p

- Conant N F *et al* *Manual of Clinical Mycology* 2nd ed Philadelphia
Saunders 1954 p 390-411
- Gilman J C *A Manual of Soil Fungi* 2nd ed Ames Iowa State College Press
1957 450 p
- Lewis C M *et al* *An Introduction to Medical Mycology* 4th ed Chicago
Gr Bk Pub 1958 p 423-440
- Lodder J and Kreger Van Rij N J W *The Yeasts A Taxonomic Study*
New York Interscience 1952 713 p
- Raper A B Thom C and Fennel D I *A Manual of the Pencillia* Baltimore
Williams and Wilkins 1949 675 p
- Thom C and Raper A B *A Manual of the Aspergilli* Baltimore Williams
and Wilkins 1945 373 p

